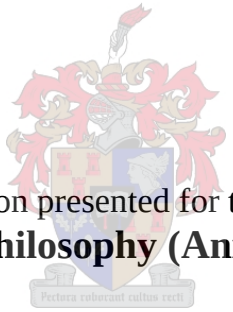
Feed supplement and meat preservative potential of grape pomace (*Vitis vinifera*) in lamb production

By

Obert Chenjerayi Chikwanha



Dissertation presented for the degree of
Doctor of Philosophy (Animal Sciences)

at

Stellenbosch University
Department of Animal Sciences, Faculty of AgriSciences

Supervisor: Dr. Cletos Mapiye
Co-supervisor: Prof. Voster Muchenje

April 2019

Declaration

By submitting this dissertation electronically, I declare that the entirety of the work contained therein is my own, original work, that I am the sole author thereof (save to the extent explicitly otherwise stated) that reproduction and publication thereof by Stellenbosch University will not infringe any third-party rights and that I have not previously in its entirety or in part submitted it for obtaining any qualification.

This dissertation includes one review article and three research articles published in peer-reviewed journals, and one article was submitted for publication and one article was being prepared for publication at the time of examination. The production of these articles was my primary responsibility and co-authored by my supervisors, as indicated below the relevant research chapters.

Date: 03 December 2018

Summary

The objective of the overall study was to evaluate the potential of grape (*Vitis vinifera* L.) pomace (GP) as a feed supplement and meat preservative in lamb production. Pomace from two red grape (Pinotage and Shiraz) varieties and one white grape variety (Sauvignon Blanc), were collected from the Welgevallen cellar to evaluate the effect of drying method [sun (7 d), oven (60 °C for 72 h) and freeze] on the nutrient composition and in vitro ruminal neutral detergent fiber (ivNDF) digestibility. Overall, sun- and oven-dried Shiraz GP were characterized by a higher dry matter, crude protein, NDF, acid detergent fiber and lignin content, compared to the other drying × variety combinations. Freeze- and oven-dried Pinotage GP were characterized by the highest mineral content, compared to freeze-dried Pinotage GP that had the most comprehensive amino acid profile and highest ivNDF digestibility at 24 and 48 h for both parameters and time periods. The second study assessed the impact of drying method on the retention of bioactive compounds and biological activities of the three types of GP. Freeze-dried Sauvignon Blanc GP had the highest proanthocyanidin content and exhibited the highest antioxidant activity relative to other drying × variety combinations. Overall, freeze-dried Shiraz GP had the most comprehensive fatty acid profile and highest phenolic content compared to other treatment combinations. Based on the nutrient composition, the low proanthocyanidin content, the availability and the fact that it is a unique South African cultivar, Pinotage GP was selected for subsequent trials. The effect of supplementing sun-dried Pinotage GP on nutrient utilization in finisher lambs was evaluated. Twenty-one Dohne Merino lambs [51.6 ± 4.70 kg body weight (BW)] were randomly assigned to a control diet group (no GP), and two treatments groups containing 100g and 200 g GP/kg diet dry matter (DM), respectively. Increasing the inclusion level of Pinotage GP in the lamb finisher diet resulted in a linear decline in carbohydrate intake, total volatile fatty acid concentration, microbial nitrogen (N) yield, and total purine derivatives excreted. No adverse effects on retention and efficiency of N utilization were observed with the GP-containing diets. A follow up study assessed

the effect of sun-dried Pinotage GP supplementation in lamb diets on growth performance, and carcass traits and meat quality attributes. Forty Dohne Merino lambs (32 ± 1.7 kg BW) were randomly assigned to five experimental diets that comprised a control (no GP), and four GP-based diets consisting of 50, 100, 150 and 200 g GP/ kg, respectively. Dry matter intake (DMI), average daily gain (ADG) and carcass traits for lambs were influenced by the addition of GP without negatively affecting meat quality traits. The optimum inclusion levels of GP for DMI and ADG were observed at 113g/kg and 96 g/kg, respectively. The last experiment evaluated the sensory quality and shelf life of retail displayed meat of lambs fed increasing levels (i.e., 0, 50, 100, 150 and 200 g GP/kg). Overall, the 150 and 200 g GP/kg diets resulted in higher antioxidant activity, and reduced lipid and protein oxidation occurring during retail display, compared to the 0 to 100 g GP/kg diets. Microbial loads increased during the retail display, but at a lower rate for the 150 and 200 g GP/kg diets. Diet had no negative organoleptic effect on the sensory quality of the lamb. Overall, the inclusion of GP improved production and shelf life without compromising meat physicochemical and sensory qualities. Based on the experimental evidence, it can be concluded that GP can be used as an alternative supplement and meat preservative by the red meat industry.

Opsomming

Die doelwit van die algehele studie was om die potensiaal van druiwe (*Vitis vinifera* L.) vrugtepulp (GP) as 'n voedingsaanvulling en vleisbeskermingsmiddel in lamsvleis produksie te evalueer. Die vrugtepulp twee rooi druif kultivars (Pinotage en Shiraz) en een wit druif kultivar (Sauvignon Blanc) is verkry van die Welgevallen kelder om die effek van droogmetode [son (7 d), oond (60 °C vir 72 h) en vries] op die voedingstofsamestelling en in vitro vertering van ruminale neutrale detergent vesel (ivNDF) te evalueer. In die geheel, is son- en oondgedroogde Shiraz-GP gekenmerk deur 'n hoër droë materiaal, ruproteïen, NDF, suur-detergent vesel en lignieninhoud, in vergelyking met die ander droogmetode × kultivar kombinasies. Vries- en oondgedroogde Pinotage-GP is gekenmerk deur die hoogste mineraalinhoud, in vergelyking met die vriesdroogde Pinotage-GP wat die mees omvattende aminosuurprofiel gehad het en die hoogste IVNDF verteerbaarheid op 24 en 48 uur vir beide parameters en tydperke getoon het. Die tweede studie het die impak van droogmetode op die behoud van bio-aktiewe verbindings en biologiese aktiwiteit van die drie soorte GP ondersoek. Gevriesdroogde Sauvignon Blanc GP het die hoogste pro-antosianidieninhoud gehad en het die hoogste antioksidant aktiwiteit getoon, relatief tot die ander droogmetode × kultivar kombinasies. In die geheel het gevriesdroogde Shiraz GP die mees omvattende vetsuurprofiel en die hoogste fenoliese inhoud in vergelyking met ander behandelingssamestellings gehad. Gebaseer op die voedingstofsamestelling, die lae pro-antosianidieninhoud, die beskikbaarheid en die feit dat dit 'n unieke Suid-Afrikaanse kultivar is, is Pinotage-GP gekies vir verdere proewe. Die effek van die aanvulling van die songedroogde Pinotage GP op die voedingstof metabolisme van afrond lammers is evalueer. Een-en-twintig Dohne Merino-lammers [51.6 ± 4.70 kg liggaamsgewig (BW)] is ewekansig toegewys aan 'n kontrole-dieetgroep (geen GP) en twee behandelingsgroepe, wat onderskeidelik 100 g en 200 g GP/kg dieet droë materiaal (DM) bevat het. Die verhoging van die insluitingsvlak van Pinotage GP in die lam afrond dieet het gelei tot 'n lineêre afname in koolhidraat inname, totale vlugtige

vetsuur konsentrasie, mikrobiese stikstof (N) opbrengs en totale purien afgeleides. Geen nadelige effekte op behoud en doeltreffendheid van N-benutting is waargeneem met die GP-bevattende dieet nie. 'n Opvolgstudie het die effek van son-gedroogde Pinotage-GP-aanvulling in lam diëte beoordeel op grond van groeiprestasie, karkas eienskappe en vleiskwaliteit. Veertig Dohne Merino-lammers (32 ± 1.7 kg BW) is ewekansig toegewys aan vyf eksperimentele diëte wat bestaan uit 'n kontrole dieet (geen GP) en vier GP-gebaseerde diëte wat onderskeidelik die insluiting van 50g, 100g, 150g en 200 g GP/kg behels het. Droë materiaal inname (DMI), gemiddelde daaglikse gewigstoename (ADG) en karkas eienskappe vir lammers is beïnvloed deur die insluiting van GP, sonder om vleisgehalte eienskappe negatief te beïnvloed. Die optimum insluiting vlakke van GP vir DMI en ADG is waargeneem teen onderskeidelik 113g/kg en 96g/kg insluitingsvlakke. Die laaste eksperiment het die sensoriese kwaliteit en die rakleeftyd van kleinhandelsnitte van lammers evalueer wat diëte aangevul met toenemende vlakke (d.w.s. 0g, 50g, 100g, 150g en 200 g GP/kg) ontvang het. In die geheel het die 150g- en 200 g GP/kg diëte tot hoër anti-oksidante aktiwiteit gelei, en verminderde lipied- en proteïen-oksidasie tydens kleinhandel vertoon, in vergelyking met die 0g tot 100 g GP/kg dieet. Mikrobiese tellings het gedurende die kleinhandel vertoontydperk toegeneem, maar teen 'n laer tempo vir die 150g- en 200 g GP/kg diëte. Dieet het geen negatiewe organoleptiese effek op die sensoriese kwaliteit van die lamsvleis gehad nie. Die oorhoofse studie het bevind dat die insluiting van GP tot verbeterde produksie en rakleeftyd bygedra het sonder om die vleis se fisiese en sintuiglike eienskappe te benadeel. Op grond van die eksperimentele bewyse kan daar tot die gevolgtrekking gekom word dat GP as 'n alternatiewe aanvulling en vleisbeskermende middel deur die rooivleisbedryf gebruik kan word.

This dissertation is dedicated to my wife and soulmate, Morongua…

and, to Fazeela, the best daughter I can imagine. You have been a gift from the beginning.

Biographical sketch

Obert Chenjerayi Chikwanha was born in a rural village of Nyahoni (Chivhu, Zimbabwe). In the early years of his childhood, he was presented with firsthand experience of handling livestock before attending primary school in Harare (Zimbabwe). He completed his high school in Bulawayo (Zimbabwe) before enrolling for a Bachelor of Science Honors degree in Agricultural Sciences (Animal Sciences; August 2001- July 2004) and a Master of Science degree in Animal Sciences (August 2004 – July 2006) at the University of Zimbabwe. For his Master's research, he evaluated the potential of local feed resources as alternative protein sources for indigenous pig in a semi-arid smallholder farming areas of Zimbabwe. He worked for the University of Zimbabwe as a temporary lecturer (February – August 2008). He later migrated to South Africa where he worked as a High school teacher (Nkotwane Secondary School) in Limpopo (South Africa; October 2008 – December 2014). In 2015, he enrolled for his PhD studies in Animal Sciences at Stellenbosch University. The main focus of his PhD study was to evaluate the potential of grape pomace, a winery byproduct, as feed ingredients and meat preservatives in intensive lamb production systems in South Africa.

Publications & conference presentations

Journal articles

1 **Chikwanha, O.C.**, Vahmani, P., Muchenje, V., & Mapiye, C., 2018. Nutritional enhancement of sheep meat fatty acid profile for human health and wellbeing. *Food Research International* 104, 25–38.

2 **Chikwanha, O.C.**, Raffrenato, E., Muchenje, V., Musarurwa, H.T., Mapiye, C., 2018. Varietal differences in nutrient, amino acid and mineral composition and in vitro rumen digestibility of grape (*Vitis vinifera*) pomace from the Cape Winelands vineyards in South Africa and impact of preservation techniques. *Industrial Crops and Products* 118, 30–37.

3 **Chikwanha, O.C.**, Raffrenato, E., Opara, U.L., Fawole, O.A., Setati, M.E., Muchenje, V., & Mapiye, C., 2018. Impact of dehydration on retention of bioactive profile and biological activities of different grape (*Vitis vinifera* L.) pomace varieties. *Animal Feed Science and* Technology 244, 116–127.

4 **Chikwanha, O.C.**, Muchenje, V., Nolte, J.E., Dugan, M.E.R., & Mapiye, C., 2018. Grape pomace (*Vitis vinifera* L. cv. Pinotage) supplementation in lamb diets: Effects on growth performance, carcass and meat quality. *Meat Science* 147, 6–12.

Oral conference presentations

1 **Chikwanha, O.C.**, Raffrenato, E., Musarurwa, H.T., Muchenje, V., & Mapiye, C. 2017. Effect of drying method on the nutrient composition of grape pomace from the Cape Winelands vineyards in South Africa. Golden Innovations for Sustainable Animal Agriculture, 50th Congress, Port Elizabeth, 18-21 September 2017.

Poster conference presentations

1 **Chikwanha, O.C.**, Gouws, P., Arnaud, E., Nolte, J.E., Muchenje, V., Dugan, M.E.R. & Mapiye, C. 2018. Effect of dietary red grape pomace on the oxidative stability and microbial quality of retail lamb meat. 2nd *International Scientific Conference on Food Security and Safety*, St. George's Hotel, Pretoria, 15-17 October 2018.

2 **Chikwanha, O.C.**, Raffrenato, E., Nolte, J.E., Muchenje, V. Dugan, M.E.R., & Mapiye, C. 2018. Supplementation of grape pomace in lamb diets and its effects on growth performance and meat quality. Red Meat Abattoir Association Conference and Congress, Spier Wine Farms, Stellenbosch. 13-15 June 2018.

3 **Chikwanha, O.C.**, Gouws, P., Nolte, J.E., Anaud, E., Muchenje, V. Dugan, M.E.R., & Mapiye, C. 2018. Extension of lamb meat shelf-life with antioxidant-rich red grape pomace feed supplements. Red Meat Abattoir Association Conference and Congress, Spier Wine Farms, Stellenbosch. 13-15 June 2018.

4 **Chikwanha, O.C.**, Gouws, P., Nolte, J.E., Anaud, E., Muchenje, V. Dugan, M.E.R., & Mapiye, C. 2018. Augmentation of lamb meat healthfulness and shelf life with antioxidant-rich red grape pomace. Cape Wools National Woolgrowers Association of South Africa (NWGA), National Congress 13 June 2018.

Acknowledgements

Undertaking this PhD has been a truly life-changing experience for me and it would not have been possible to do without the support and guidance that I received from many people.

Firstly, I would like to express my sincere gratitude to my supervisor Dr. C. Mapiye for the intellectual guidance, motivation, and immense knowledge. His guidance helped me in all the time of research and writing of this thesis. I could not have imagined having a better supervisor for my Ph.D. study. I have been extremely lucky to have a supervisor who cared so much about my work, and who responded to my questions and queries so promptly. I would also like to thank my co-supervisor, Prof. V. Muchenje (University of Fort Hare) for his guidance and great support and kind advice throughout my PhD study. I benefited from his advice, particularly so when exploring new ideas.

Besides my supervisor, I would like to thank Dr. M.E.R. Dugan (Lacombe Research and Development Centre, Agriculture and Agri-Food Canada), Dr. E. Raffrenato (Stellenbosch University), Dr. H.T. Musarurwa (Walter Sisulu University) and Dr. O.A. Fawole (Stellenbosch University), J.E. Nolte (Meadow Feeds, Paarl) for sharing their knowledge that influenced the work and resulted in cooperative joint publications. They all have played a major role in polishing my research writing skills.

My sincere thanks also go to Prof. U.L. Opara, Prof. P. Gouws and Dr. M.E. Setati who gave access to their laboratory facilities. Without their precious support it would not be possible to conduct this research. I would like to thank Dr. R.R. Mphahlele, Dr. E.I. Moelich, Dr. S. Erasmus and Dr. M.E. Setati who assisted me with my phytochemicals, meat sensory and ruminal microbial analyses, respectively.

I am also grateful to all the academic staff of the Department of Animal Sciences for comments and criticism that enriched my work, particular mention goes to Prof. L.C. Hoffman and Prof. K. Dzama for their scientific advice and knowledge and many insightful discussions and suggestions. I am thankful also to the Department of Animal Sciences technical staff members (Beverly, Lisa, Danie, Michael, Janine and Jonas who have been kind enough to advise and help in their respective roles. Special mention goes to Dr. H. Lambrechts for helping me with the translation of the dissertation summary. I am grateful to Mr. W. Kerwel and the workers (Kallie, Sam and James) at Welgevallen Experimental Farm who assisted me during my growth and digestibility trials.

I thank my colleagues and friends in the Department of Animal Sciences for the encouragement, constructive criticisms and assistance they offered to me throughout my period of study. I will forever be grateful to Tawanda T., Trust and Tatenda for the help they gave me from the collection of grape pomace, feeding of lambs, all the lab work and proof reading my work. My completion of this project could not have been accomplished without the support of my fellow postgraduate students, Faith, Tawanda M., Tautiko, Gregory, Megan, Daniel, Raoul, Sonya, Aimee, Fundeka, Chido, Leo, Zikhona, Nwabisa and Stellenbosch SARCHi Meat Science students. I would also like to thank George, Tony and Sipokazi for proof reading my dissertation.

I gratefully acknowledge the funding sources that made my Ph.D. work possible. I was funded by the Research and Technology Funds from National Research Foundation (RTF-NRF) Grant No: 98700 and Cape Wools South Africa Grant No: S005139. Special thanks to goes to Stellenbosch University Welgevallen Cellar and Bellevue Wine Estate who provided me with grape pomaces, Meadow feeds for the formulations and mixing of all the feeds and Tomis abattoir (Hermon) for the use of their slaughter facilities and staff that assisted during the slaughter procedures.

I would like to thank my siblings and their families, my in-laws and my extended family. They gave me enough moral support, encouragement and motivation to accomplish the personal goals. I consider myself nothing without them. Special mention goes to my brothers, Tapiwa, and Obey and their family who have always supported me financially so that I only pay attention to the studies and achieving my objective without any obstacle on the way.

To my late parents, Oddwell and Alice, thank you for the principles and values you instilled in me. I know you will be proud of me. Had it not been for my father's unflinching insistence and support, my dreams of excelling in education would have remained mere dreams. I thank you both with all my heart and I know you're up there, listening, watching over me and sending me blessings constantly. To my second parents, Henry and Loice Madimu, you guided me for as long as I can remember. I would not have been where I am today and what I am today. I am at a loss for words but "thank you".

Most importantly, I wish to thank my loving and supportive wife, Morongua, and our daughter, Fazeela, for all of the sacrifices and unending inspiration that you've made on my behalf. I cannot find any good words to express my feelings except saying "thank you so much".

Preface

This dissertation is presented as a compilation of eight chapters. Each chapter is introduced separately and is written according to the style of the Animal Feed Science and Technology journal. Part of the literature review (Chapter 2) was published in Food Research International journal. Chapter 3 was published the Industrial Crops and Products journal, Chapter 4 was published in Animal Feed Science and Technology journal. Chapter 5 was submitted for publication in Small Ruminant Research journal. Chapter 6 was published in Meat Science journal and Chapter 7 is under preparation for publication in the Meat Science journal. As each chapter has been written as an individual entity, some repetition between chapters is unavoidable. The research chapters are prefaced by a summary of research performed, general introduction of the topic, culminating in a general discussion and conclusion of the project. Language, style and referencing are in accordance with specifications of the journal Animal Feed Science and Technology. The opinions expressed, and conclusions arrived at in this study are those of the author and are not necessarily to be attributed to the National Research Foundation, Cape Wools, Meadow Feeds or the Department of Animal Sciences, Stellenbosch University.

Chapter 1	**General introduction, project objectives and hypotheses**
Chapter 2	**Literature review** Potential utilization of grape pomace to improve sheep production and meat quality
Chapter 3	**Research results** Impact of preservation technique on nutrient composition and in vitro ruminal digestibility of pomace from three grape varieties
Chapter 4	Impact of dehydration on retention of bioactive compounds and biological activities of different grape (*Vitis vinifera* L.) pomace varieties
Chapter 5	Effect of grape (*Vitis vinifera* L.) pomace supplementation on nutrient utilization in finisher lambs
Chapter 6	Grape pomace (*Vitis vinifera* L. cv. Pinotage) supplementation in lamb diets: Effects on growth performance, carcass and meat quality
Chapter 7	Shelf life stability and eating quality of grape pomace enhanced lamb meat
Chapter 8	**General discussion, Conclusions and recommendations**

Table of Contents

List of Figures

List of Tables

List of Abbreviations

a*	Redness
ADFom	Acid detergent fiber expressed exclusive of residual ash
ADL	Acid detergent lignin
ADG	Average daily gain
AHDB	Agriculture and Horticulture Development Board
aNDFom	NDF assayed with a heat stable amylase and expressed exclusive of residual ash
ANOSIM	Analysis of similarity
ARISA	Automated Ribosomal Intergenic Spacer Analysis
C	Chroma
CFU	Coliform forming units
CP	Crude protein, being total N × 6.25
CSIRO	Commonwealth Scientific and Industrial Research Organization
cv	Cultivar
DAFF	Department of Agriculture, Forestry and Fisheries
DM	Dry matter
DMI	Dry matter intake
DNA	Deoxyribonucleic acid
DNPH	2,4-dinitrophenylhydrazine
DOMI	Digestible organic matter intake
DPPH	2,2-diphenyl-1-picrylhydrazyl
EE	Ether extract
FA	Fatty acids
FAO	Food and Agriculture Organization
FAOSTAT	Food and Agriculture Organization Corporate Statistical Database
FCE	Food conversion efficiency
FRAP	Ferric reducing antioxidant power
g/ kg	grams per kilogram
GP	Grape pomace
H	Hue angle
IMF	Intramuscular fat
ivNDF	in vitro neutral detergent fiber digestibility
L*	Lightness
Lignin (sa.)	Lignin determined by solubilization of cellulose with sulfuric acid
LSMEANS	Least square means
LTL	*Longissimus thoracis et lumborum*
MDA	Malondialdehyde
mg/ kg	milligrams per kilogram
mg/ mL	milligrams per milliliter
MJ/ kg	megajoules per kilogram
mL	Milliliter
mM	Millimolar
MUFA	Monounsaturated fatty acids
N	Nitrogen

n-3	Omega 3
n-3: n-6	omega 3 to omega 6 ratio
n-6	Omega 6
NDF	Neutral detergent fiber
NH3-N	Ammonia nitrogen
nm	Nanometer
OECD	Organization for Economic Co-operation and Development
OM	Organic matter
P:S	Polyunsaturated fatty acids to saturated fatty acids ratio
PCA	Principal component analysis
PD	Purine derivatives
PUFA	Polyunsaturated fatty acids
RSA	Radical scavenging activity
RSEG	Response surface regression
SAS	Statistical Analysis Systems
SFA	Saturated fatty acids
TBARS	Thiobarbuturic acid reactive substances
TPC	Total phenolic content
TVC	Total viable counts
VFA	Volatile fatty acids
WBSF	Warner-Braztler shear force
WCW	Warm carcass weight
µL	Microliter

Chapter 1 General introduction

1.1 Background

Historically, sheep have been reared for wool production with meat being a secondary enterprise, but with the decline in wool price there has been a surge in lamb production (Cloete and Olivier, 2010). In South Africa, sheep farming is more concentrated in the Cape provinces and mainly relies on natural pastures (Cloete and Olivier, 2010). This poses risks on production because of the fluctuating quantity and quality of feed supply throughout the year (Meissner, 1997; Thornton et al., 2009). The bulk of the sheep meat produced in South Africa is consumed locally; however, the country still remains a net importer of lamb meat (Cloete and Olivier, 2010; Meissner et al., 2013). Notwithstanding these challenges, local and global lamb meat prices continue to increase (Cloete and Olivier, 2010; Ferguson et al., 2014; Gracia and De-Magistris, 2013). For these reasons, sheep producers are shifting to fattening their animals in feedlots (Brand et al., 2013; Meissner et al., 2013), which currently supply the largest proportion of lamb that reaches retail shelves in South Africa (Brand et al., 2013). Feed supply in feedlots is a challenge as it can constitute 70% of lamb feedlot production costs (Makkar, 2016).

In South Africa, feedlot diets primarily consist of cereals (e.g., maize, barley and wheat) as energy sources and leguminous oil seed cakes (e.g., soybean meal and sunflower seed cake) as protein and polyunsaturated fatty acids (PUFA) sources (Brand et al., 2017; Nkosi and Meeske, 2010). The PUFA content in meat plus the high level of protein and heme pigments are a major cause of meat oxidation. The oxidative processes and microbial contamination from slaughter to point of sale contributes to meat discoloration, rancidity and spoilage during storage, which and leads to the production of toxic compounds that can be harmful to human health (Cunha et al., 2018; Papuc et al., 2017). The effects are

more noticeable in the traditional retail polyvinyl chloride film overwrap display which has a shelf life of between five to seven days (Soldatou et al., 2009).

Over the years, researchers and industry partners have developed several innovative technologies to counteract meat spoilage, these include chemical preservatives, refrigeration, high hydrostatic pressure, irradiation, dehydration and active packaging (Chen et al., 2012; Zhou et al., 2010). However, due to the cost of some of these technologies, industry mostly use chemical preservatives, such as synthetic antioxidants and/ or antimicrobials for the preservation of fresh meat (Troy et al., 2016). There is an increasing demand by consumers for the use of natural ingredients in food production because of the potential adverse effects of synthetic preservatives on consumer health (Brewer, 2011; Cunha et al., 2018). These natural sources of preservatives include fruit byproducts, which contain bioactive phytochemicals (Brewer, 2011; Papuc et al., 2017).

South Africa has a diversity of fruits, which produce byproducts that are either underutilized or discarded as waste. Of these, grapes are one of the most economically important fruit crops with approximately 1.5 million tons produced annually (SAWIS, 2017), of which 25% of the pressed grapes becomes pomace (Yu and Ahmedna, 2013). Grape pomace (GP) is a winery byproduct comprising of seeds, skins and stalks (Zhang et al., 2017). It has an interesting nutrient profile; for example, it is rich in PUFA high in dietary fiber, it has a valuable array of bioactive compounds such as amino acids, vitamins, minerals and proanthocyanidins (Baumgärtel et al., 2007; Brewer, 2011; García-Lomillo and González-SanJosé, 2017). During wine-making, large quantities of GP are produced and pose potentially severe environmental pollution problems and also have economic costs to the winery through transportation to landfills (Beres et al., 2017). Therefore, it is important to find sustainable ways of reutilization of this GP so as to and alleviate some of the challenges posed by its disposal.

The use of locally-bred red grape cultivars such as Pinotage in feedlot diets can be an effective way of improving lamb performance and extending meat shelf life. Little is, however, known about the meat production and preservative capabilities of GP. Although GP contains high levels of proanthocyanidins, when fed at moderate levels (20 – 50 g/ kg DM) they are beneficial to ruminants (Piluzza et al., 2014) because of their antioxidative and antimicrobial properties (Guerra-Rivas et al., 2016). The nutrient-binding properties of proanthocyanidins protect degradation of proteins and increase rumen undegradable protein, which can thus be absorbed post-ruminally and subsequently improve animal growth (Piluzza et al., 2014). Furthemore, proanthocyanidins can improve meat yield and quality because of their preservative (antioxidant and antimicrobial) properties (Guerra-Rivas et al., 2016; Manso et al., 2016). Various authors (Cunha et al., 2018; Guerra-Rivas et al., 2016; Muela et al., 2014) have reported that dietary phenolics improve the shelf life of meat in terms of meat color, lipid and protein oxidation, and inhibition of microbial growth inhibition.

The scant documentation on the positive effects of GP on animal performance, meat quality and shelf stability is mainly associated with a rather scarce fundamental knowledge about their chemical composition and suitable preservation method for long-term use. In addition, there is no data on the sensory quality of lamb meat enriched with GP. There is, therefore, a knowledge gap for optimum utilization of this locally available and cheap source of polyphenols in feedlot feeding of lambs, especially in the Cape Winelands regions of South Africa where GP is found in abundance.

1.2 Justification

Intensive lamb production in South Africa is challenged by unavailability and high cost of feed (Nkosi and Meeske, 2010) as well as high postharvest losses caused by oxidation and microbial spoilage (Buys et al., 2000). Overall, winery byproducts are promising cheaper sources of fiber, PUFA, phytochemicals,

thus, offering new commercial opportunities to the animal feed and meat industries. However, there are few, if any, studies in South Africa detailing nutritional and phytochemical profiles of GP and how they influence animal production, shelf life and sensory quality of lamb meat. Use of GP as a dietary supplement will reduce costs of conventional ingredients used as food sources for humans but will also improve safety, shelf life and healthfulness of meat. It will also reduce meat losses and meat-borne illnesses associated with discoloration, rancidity and microbial spoilage. Reducing meat losses along the lamb supply chain has a potential to contribute to improved food and nutrition security at household and national levels. Utilization of GP also benefits the winery industry to reduce economic costs and ecological costs related to its disposal. In addition, the current research has the potential to address societal demands for livestock products conforming to the concepts of "clean", "green" production and 'healthy'.

1.3 Objectives

The broad objective of the current study was to evaluate the potential of grape pomace as a feed supplement and meat preservative in lamb production under feedlot conditions in the Cape Winelands region of South Africa. The specific objectives were to:

1. Evaluate the impact of dehydration method and grape (*Vitis vinifera* L.) variety on the chemical composition and *in vitro* ruminal neutral detergent fiber digestibility of grape pomace;
2. Assess the impact of dehydration method on retention of bioactive compounds and bioactivities of different grape (*Vitis vinifera* L.) pomace varieties;
3. Investigate the effect of supplementing increasing levels of grape (*Vitis vinifera* L. cv. Pinotage) pomace on nutrient utilization in finisher lambs;

4. Determine the potential of feeding increasing levels of grape (*Vitis vinifera* L. cv. Pinotage) pomace on growth performance and carcass and meat quality of finisher lambs and;
5. Assess the effect of feeding increasing levels of grape (*Vitis vinifera* L. cv. Pinotage) pomace on the shelf life stability and sensory quality of lamb meat.

1.4 Hypotheses

The alternative hypotheses tested were:

1. The nutrient composition and *in vitro* ruminal neutral detergent fiber digestibility of grape pomace varieties dried using sun-, oven- and freeze-drying are different;
2. Grape pomace varieties dried using sun-, oven- and freeze-drying have different retention of bioactive compounds and biological activities;
3. Increasing the level of grape (*Vitis vinifera* L. cv. Pinotage) pomace improves nutrient utilization in finisher lambs;
4. Supplementing grape (*Vitis vinifera* L. cv. Pinotage) pomace improves growth performance, carcass traits and meat quality characteristics of finisher lambs;
5. Supplementing grape (*Vitis vinifera* L. cv. Pinotage) pomace to finisher lambs improves the meat shelf stability and its sensory quality.

1.5 References

Baumgärtel, T., Kluth, H., Epperlein, K., Rodehutscord, M., 2007. A note on digestibility and energy value for sheep of different grape pomace. Small Rumin. Res. 67, 302–306.

Beres, C., Costa, G.N.S., Cabezudo, I., da Silva-James, N.K., Teles, A.S.C., Cruz, A.P.G., Mellinger-

Silva, C., Tonon, R.V., Cabral, L.M.C., Freitas, S.P., 2017. Towards integral utilization of grape pomace from winemaking process: A review. Waste Manag. 68, 581–594.

Brand, T.S., Genis, M.P., Hoffman, L.C., van de Vyver, W.F.J., Swart, R., Jordaan, G.F., 2013. The effect of dietary energy and the inclusion of a β-adrenergic agonist in the diet on the meat quality of feedlot lambs. S. Afr. J. Anim. Sci. 43, S140–S145.

Brand, T.S., Van Der Westhuizen, E.J., Van Der Merwe, D.A., Hoffman, L.C., 2017. Effect of days in feedlot on growth performance and carcass characteristics of Merino, South African Mutton Merino and Dorper lambs. S. Afr. J. Anim. Sci. 47, 26–33.

Brewer, M.S., 2011. Natural antioxidants: Sources, compounds, mechanisms of action, and potential applications. Compr. Rev. Food Sci. Food Saf. 10, 221–247.

Buys, E.M., Nortjé, G.L., Jooste, P.J., Von Holy, A., 2000. Combined effect of modified atmosphere bulk packaging, dietary vitamin E supplementation and microbiological contamination on colour stability of Musculus gluteus medius. Meat Sci. 55, 403–411.

Chen, J.H., Ren, Y., Seow, J., Liu, T., Bang, W.S., Yuk, H.G., 2012. Intervention technologies for ensuring microbiological safety of meat: Current and future trends. Compr. Rev. Food Sci. Food Saf. 11, 119–132.

Cloete, S.W., Olivier, J.J., 2010. South African sheep and wool Industry, in: International Sheep and Wool Handbook. pp. 95–112.

Cunha, L.C.M., Monteiro, M.L.G., Lorenzo, J.M., Munekata, P.E.S., Muchenje, V., de Carvalho, F.A.L., Conte-Junior, C.A., 2018. Natural antioxidants in processing and storage stability of sheep and goat meat products. Food Res. Int. 111, 379–390.

Ferguson, D.M., Schreurs, N.M., Kenyon, P.R., Jacob, R.H., 2014. Balancing consumer and societal

requirements for sheep meat production: An Australasian perspective. Meat Sci. 98, 477–483.

García-Lomillo, J., González-SanJosé, M.L., 2017. Applications of wine pomace in the food industry: Approaches and functions. Compr. Rev. Food Sci. Food Saf. 16, 3–22.

Gracia, A., De-Magistris, T., 2013. Preferences for lamb meat: A choice experiment for Spanish consumers. Meat Sci. 95, 396–402.

Guerra-Rivas, C., Vieira, C., Rubio, B., Martínez, B., Gallardo, B., Mantecón, A.R., Lavín, P., Manso, T., 2016. Effects of grape pomace in growing lamb diets compared with vitamin E and grape seed extract on meat shelf life. Meat Sci. 116, 221–229.

Makkar, H.P.S., 2016. Animal nutrition in a 360-degree view and a framework for future R&D work: Towards sustainable livestock production. Anim. Prod. Sci. 56, 1561–1568.

Manso, T., Gallardo, B., Guerra-Rivas, C., 2016. Modifying milk and meat fat quality through feed changes. Small Rumin. Res. 142, 31–37.

Meissner, H.H., 1997. Recent research on forage utilization by ruminant livestock in South Africa. Anim. Feed Sci. Technol. 69, 103–119.

Meissner, H.H., Scholtz, M.M., Palmer, A.R., 2013. Sustainability of the South African livestock sector towards 2050 Part 1: Worth and impact of the sector. S. Afr. J. Anim. Sci. 43, 282–297.

Muela, E., Alonso, V., Campo, M.M., Sañudo, C., Beltrán, J.A., 2014. Antioxidant diet supplementation and lamb quality throughout preservation time. Meat Sci. 98, 289–295.

Nkosi, B.D., Meeske, R., 2010. Effects of ensiling totally mixed potato hash ration with or without a heterofermentative bacterial inoculant on silage fermentation, aerobic stability, growth performance and digestibility in lambs. Anim. Feed Sci. Technol. 161, 38–48.

Papuc, C., Goran, G.V., Predescu, C.N., Nicorescu, V., Stefan, G., 2017. Plant polyphenols as antioxidant

and antibacterial agents for shelf-life extension of meat and meat products: Classification, structures, sources, and action mechanisms. Compr. Rev. Food Sci. Food Saf. 16, 1243–1268.

Piluzza, G., Sulas, L., Bullitta, S., 2014. Tannins in forage plants and their role in animal husbandry and environmental sustainability: A review. Grass Forage Sci. 69, 32-48.

SAWIS, 2017. South African Wine Industry Statistics Nr. 42. http://www.sawis.co.za/info/annualpublication.php (accessed 10.18.18)

Soldatou, N., Nerantzaki, A., Kontominas, M.G., Savvaidis, I.N., 2009. Physicochemical and microbiological changes of "Souvlaki"- A Greek delicacy lamb meat product: Evaluation of shelf-life using microbial, colour and lipid oxidation parameters. Food Chem. 113, 36–42.

Thornton, P.K., van de Steeg, J., Notenbaert, A., Herrero, M., 2009. The impacts of climate change on livestock and livestock systems in developing countries: A review of what we know and what we need to know. Agric. Syst. 101, 113–127.

Troy, D.J., Ojha, K.S., Kerry, J.P., Tiwari, B.K., 2016. Sustainable and consumer-friendly emerging technologies for application within the meat industry: An overview. Meat Sci. 120, 2–9.

Yu, J., Ahmedna, M., 2013. Functional components of grape pomace: Their composition, biological properties and potential applications. Int. J. Food Sci. Technol. 48, 221–237.

Zhang, N., Hoadley, A., Patel, J., Lim, S., Li, C., 2017. Sustainable options for the utilization of solid residues from wine production. Waste Manag. 60, 173–183.

Zhou, G.H., Xu, X.L., Liu, Y., 2010. Preservation technologies for fresh meat - A review. Meat Sci. 86, 119–128.

Chapter 2 Potential utilization of grape pomace to improve sheep production and meat quality[1]

2.1 Introduction

Southern Africa is predicted to become drier, with average temperatures expected to rise by between 1.5 and 2ºC (Meissner et al., 2013). The effects will be greater for water scarce countries such as South Africa. The agricultural sector will be the hardest hit, especially towards the western part of the country where sheep production is concentrated (Cloete and Olivier, 2010). Recurrent and frequent droughts in these areas hamper the full potential of this sector considering that South Africa is a net importer of lamb meat (Cloete and Olivier, 2010). The challenges faced by producers in these dry areas include severe nutritional deficits, disease and health problems, stock theft and predation (Ben Salem, 2010; Cloete and Olivier, 2010). However, in the past few years, many producers have shifted to rounding off lambs under intensive production systems (i.e., feedlotting) for 4 to 6 weeks (Brand et al., 2013). Finishing lambs in

[1] A part of this chapter has been published:

Chikwanha, O.C., Vahmani, P., Muchenje, V., Dugan, M.E.R., Mapiye, C., 2018. Nutritional enhancement of sheep meat fatty acid profile for human health and wellbeing. Food Res. Int. 104, 25–38. doi:10.1016/j.foodres.2017.05.005

feedlots tends to be more opportunistic, as it is largely influenced by the price of feed grain, high growth rates and the high mutton and lamb prices (Brand et al., 2013). However, the challenge with feedlotting is the high cost of feed, which can be over 70% of the total production costs and can drastically affect profit margins (McGrath et al., 2018). This challenge is further exacerbated by the demand for cereal grains, which compete for human consumption.

The intensification of sheep production to curb certain environmental threats, such as forage inadequacies often result in unintended consequences. For instance, the spoilage of raw meat represents a significant loss to the industry, which could be as high as 40% of total production, mainly because of oxidative processes and microbial contamination (Sun and Holley, 2012). These oxidative processes in meat are similar for all biomolecules (i.e., lipids, proteins and heme pigments) and occur concurrently (Cunha et al., 2018). Furthermore, the dense nutrient composition and high-water activity of meat also influences its deterioration through microbial growth (Sun and Holley, 2012). These biochemical process and microbial spoilage lead to undesirable changes to the meat including meat discoloration, development of rancidity, loss of nutritional value and the production of potentially toxic compounds that can be harmful to human health (Papuc et al., 2017; Sun and Holley, 2012). Overall, all this has a negative impact on the shelf life of meat and consumer appeal and satisfaction.

Shelf life is defined as the period of time between packaging of a product and its end use while the product properties remain acceptable for the product user (Lorenzo and Gómez, 2012). To maintain acceptable meat quality, several synthetic preservatives are currently in use. However, these are associated with microbial resistance and have potential health hazards (Pisoschi et al., 2018). In addition to these concerns, consumer trends have forced the industry to search for alternative sources of preservatives, which are considered more active and safer than synthetics (Cunha et al., 2018; Pisoschi

et al., 2018). These alternative sources include natural preservatives, which can be used to address issues related to microbial resistance, negative effects of synthetics on human health and assurance of meat safety and quality. Direct application of these natural preservatives on meat could be a viable option but they could alter the sensory quality. Therefore, dietary supplementation is a better alternative because research has shown that the active compounds from some fruit byproducts, such as tomato, grape, olive and citrus, can be deposited in muscle upon digestion (Inserra et al., 2014; Luciano et al., 2009; Ortuño et al., 2015), subsequently improving meat shelf life.

Current studies have proven that bioactive compounds from fruit byproducts have antioxidative and antibacterial properties due to their higher levels of residual phytochemicals (García-Lomillo and González-SanJosé, 2017). This would reduce their negative effects on the environment and improve economic returns since feeding these residues to livestock is an efficient way to upgrade low quality materials into high quality foods (Kasapidou et al., 2015). However, there is a gap in knowledge for optimum utilization of many locally produced fruit byproducts in feedlot systems. Use of winery byproducts in ruminant diets in wine-producing developing countries is scant. In South Africa for example, there is little if any information on the use of pomace from local grape (*Vitis vinifera* L.) varieties in ruminant diets, more so, the locally bred cultivar, Pinotage. Grapes are amongst the most produced horticultural fruits in South Africa generating large quantities of waste. Grape pomace (GP) is a complex lignocellulosic material from the wine industry and has been undervalued because of its low economic value (Manso et al., 2016). Yet, it is a potential cheap and abundant source of phytochemical compounds (García-Lomillo and González-SanJosé, 2017). Its underutilization as a novel ruminant feed is associated with scarce fundamental knowledge about the chemical composition and presence of anti-quality factors. Furthermore, there is limited information on the positive effects of GP as an alternative

ingredient in ruminant diets with regards to animal performance and end product quality. This current review gives an overview of GP as a functional feed ingredient in farm animal nutrition and its effect on ruminal parameters, growth performance and meat quality.

2.2 Global sheep production and consumption patterns

The global sheep population in 2014 was 1.2 billion with the largest numbers in Asia, followed by Africa (FAOSTAT, 2016). Sheep production patterns for selected countries are shown in Table 2.1, with China leading at 16%, followed by Australia (6%), India (5%), Iran (4%), and Nigeria, Sudan, Turkey and UK at 3% each (FAOSTAT, 2016). The OECD (2016) report predicts that annual increase in global sheep production is expected to increase by 2.1% with the bigger contribution coming from China, Pakistan, Sudan and Australia. Despite this forecast, sheep production still lags behind other livestock species such as beef, pork and poultry, respectively (OECD, 2016). The production of livestock is multifactorial, as such, is driven by population growth, urbanization, increasing incomes, among other factors (Montossi et al., 2013). In developed countries, sheep production has stagnated because of the preference to beef (McAfee et al., 2010).

In terms of global sheep meat production, 8.9 million tons were produced in 2015 with developing countries contributing 80% of this share (Table 2.1; FAOSTAT, 2016). Historically, sheep meat was regarded as a secondary product to wool production. However, the decline in wool production resulted in a surge of sheep meat production and consumption, a trend which is predicted to continue (Montossi et al., 2013). The consumption patterns vary greatly among regions with as low as 0.7 kg per capita in North America and 25 times higher in the Oceania region (i.e., 17 kg) (Montossi et al., 2013). Globally, the average consumption is about 1.7 kg per capita annually (OECD, 2016). China also dominates in terms of sheep consumption (46% of the global share) followed by India at 27% (AHDB, 2016). The

high consumption in China is driven by consumer diversification as they shift from pork to beef and sheep meat, in addition to the growing middle class, who have more buying power (Mao et al., 2016). In regions such as North Africa, the Middle East, India and parts of Europe, sheep meat is a primary source of animal protein due to religious and culinary backgrounds (Sañudo et al., 2013). Australia and New Zealand, the leading global sheep meat exporters, are also major consumers of sheep meat (Montossi et al., 2013; Sañudo et al., 2013).

Global meat consumption among livestock species follows a similar trend to production statistics. The reasons for low sheep meat consumption patterns in most countries include high lamb price (Brand et al., 2013), preference for fish in countries such as Japan and Norway, and issues related to health and food safety concerns associated with red meat (McAfee et al., 2010). In addition, the diverse culinary backgrounds also affect consumer perception of sheep meat consumption (Sañudo et al., 2013), while most North Americans do not eat sheep meat at all (Jones, 2004). Overall, the variation in sheep meat consumption between and within regions reflects differences in population growth and socio-economic factors. In that regard, society should not disregard consumption of sheep meat because it is a vital source of nutrients, particularly its high content of beneficial fatty acids, such as conjugated linoleic acids, which may play key roles in achieving overall health and wellness (Mapiye et al., 2012). Most consumers perceive all fatty acids in red meat as unhealthy, thus, there is a tendency to overlook the nutritional benefits.

Table 2.1 Sheep production and consumption patterns in selected countries

Country	Population (heads million)[1]	Meat production (million tons)[1]	Consumption (kg/ capita)[2]
China	194 927	2.184	2.9
Australia	72 612	0.721	8.1
India	63 000	0.235	0.5
Iran	45 000	0.148	3.3
Nigeria	41 327	0.139	2.1
Sudan	39 846	0.251	10.7
United Kingdom	33 743	0.298	1.8[3]
Turkey	31 100	0.313	4.1
Ethiopia	29 332	0.088	1.3
New Zealand	29 803	0.487	2.4
Pakistan	29 095	0.164	2.1
Algeria	27 807	0.291	7.1
South Africa	24 123	0.184	3.1
Russia	22 247	0.186	1.1
Brazil	17 614	0.086	0.4
Indonesia	16 091	0.044	0.4
Spain	15 431	0.114	1.7[4*]
Kazakhstan	15 198	0.139	8.1
Argentina	14 534	0.060	1.2
Peru	12 388	0.034	1.2

Sources:[1] FAOSTAT (2016); [2] OECD (2016); [3] (AHDB, 2015); [4] Rodríguez-Serrano et al. (2016).

2.3 Sheep production in South Africa

In South Africa 80% of the agricultural land is only suitable for extensive sheep production system (Cloete and Olivier, 2010). The Eastern Cape, Northern Cape, Free State and Western Cape in that order are the leading sheep producing provinces in South Africa (Table 2.2). Some vast areas such as the Cape and Free State provinces have experienced continuous droughts over the years which has limited forage production and, subsequently, animal production. South Africa is a net importer of sheep meat (Cloete and Olivier, 2010; Meissner et al., 2013). Apart from the challenges to sheep production, the demand for lamb meat among the emerging middle class is growing, which further puts a strain on its supply. Other factors affecting the decline of the national flock include stock theft and predation (Table 2.2) (DAFF,

2018; Meissner et al., 2013). In the North West and Mpumalanga provinces, mining operations have also negatively impacted the growth of the sheep population (Cloete and Olivier, 2010) because of an increase in pollution levels of water sources. However, feed is one of the limiting constraints to sheep production and profitability (Cloete and Olivier, 2010). In this context, more emphasis of this review will focus on how feed shortages affect sheep production and meat quality in the South African context. Furthermore, the potential use of agro-industrial by products as an alternative source of feed in sheep production will be explored.

Table 2.2 Sheep population in South Africa (per province)

Province	1996	2000	2004	2008	2012
Eastern Cape	8 260 563	8 528 604	7 722 471	7 571 170	7 294 003
Free State	6 127 400	5 672 034	5 176 957	4 945 228	4 806 186
Gauteng	113 808	85 461	92 396	106 472	104 686
KwaZulu-Natal	1 024 202	822 472	776 123	761 278	762 448
Limpopo	198 543	213 685	216 916	250 572	258 996
Mpumalanga	1 859 058	1 671 535	1 759 065	1 796 190	1 774 933
Northern Cape	7 514 051	6 634 407	6 493 882	6 204 217	6 018 088
North West	792 645	716 536	615 559	707 969	681 189
Western Cape	3 125 444	2 664 070	2 654 071	2 719 800	2 770 569
Total	**29 015 714**	**27 008 805**	**25 507 440**	**25 062 896**	**24 471 098**

Source: (DAFF, 2018).

2.3.1 Feed shortage challenges in sheep production

The reliance of sheep production on rangelands poses risks on productivity because of poor nutritional quality of the vegetation, mainly a deficiency and imbalance of minerals and low protein content during the dry season (Meissner, 1997; Thornton, 2010). Most sheep producers are thus shifting to feedlotting, not only because of their ability to manage the risks associated with fluctuating feed quantity and quality (Thornton, 2010), but feedlot-fed animals achieve higher growth rates over a shorter production cycle (Brand et al., 2013). On the contrary, feedlot-raised animals may produce fat carcasses

that could negatively affect meat quality compared to those on pastures when slaughtered at similar growth stages (Díaz et al., 2002). Furthermore, the volatility of energy prices also puts intensive production systems at risk and, consequently, affects profit margin of the enterprise (Thornton, 2010). Added to this are the high feed costs bearing in mind that approximately 33% of the 2310 million tons of global cereal grain harvest are used for livestock feed (FAO, 2013). Current research and practical experience from some farmers indicate that there are reliable and cost-effective ways of increasing profitability from ruminants by optimizing use of novel feeds from various sources, such as fruit byproducts because of the rich nutrient and phytochemical profile (Kasapidou et al., 2015).

2.3.1.1 Potential mitigation strategies

Fruit byproducts are an important feed source for ruminant animal production because they do not compete with human food. Although, they constitute an underexploited feed resource, their use has gained much attention as a means of sustainable management, which can concomitantly increase local economies profit (Naziri et al., 2014). An example is GP, which has been incorporated in ruminant diets because of its high fiber content and its relatively lower cost compared with expensive purchased lucerne or hay (Moate et al., 2014). Grape pomace is less digestible than lucerne, and in finishing systems, its inclusion lowers the dietary energy content, which can reduce growth. The adoption of fruit byproducts is hampered by the nutritional variability and presence of anti-quality factors (Makkar, 2003). Therefore, nutritionists have often excluded them in dietary formulations. Table 2.3 shows some selected studies where various fruit byproducts have been evaluated in ruminant production.

Although researchers aim to find alternative fiber cheaper feed sources, it should not be at the expense of animal productivity and meat quality. This is often the case when accurate chemical analyses of the fruit byproducts are not done, and the resultant diets compromise the proper functioning of ruminal

microflora. Subsequently, the host animal is no longer able to meet its nutritional requirements as a result of main nutrient imbalance for and/ or presence of some anti-quality factors.

2.3.2 Meat losses challenge

In South Africa, available statistics show that leading retailers lose at least 10% of their packaged fresh meat from display each week owing to spoilage caused by oxidation and microbial contamination (Buys et al., 2000). The main factors affecting meat quality are oxidation and microbial spoilage. In abattoirs meat is inspected through visual assessment, without considering microbiological tests (Rani et al., 2017). Knowledge of meat-borne microbes is critical considering that the burden emanating from these microbes is still unknown (Newell et al., 2010). Signs of spoilage, including discoloration and rancidity are poorly accepted by consumers. They result in large economic losses to retailers as the meat cannot be sold, unless at a significantly reduced price or it can either be minced and/ or discarded depending on the microbial load (Li and Liu, 2012). Oxidative processes have greater effects in meat from high concentrate-based diets compared to those on pastures, as the grass-fed tend to have high levels of antioxidants. Dietary manipulation has a significant influence on the physicochemical and organoleptic parameters of meat (Table 2.3). Priolo et al. (2002) reported that meat from feedlot-fed lambs was lighter, tenderer and juicier than meat from grass-fed lambs. This is further supported by the study of Priolo and Vasta (2007) who observed that tanniferous-based diets tend to have lighter colored meat in small ruminants. Consumers generally associate lighter colored meat with freshness, therefore, the use of tannin-based ingredients such as GP could be a beneficial factor at point of purchase.

Table 2.3 Effects of fruit byproducts on growth performance and meat quality in ruminant production

Fruit by products	Effect on growth parameters	Meat quality parameters	References
Dried citrus pulp, 300 and 400 g/ kg	DMI, ADG, FCE, WCW (⇔) DMI, ADG (⇔), FCE, WCW (⇓)	N/A	Caparra et al. (2007)
Dried grape pomace, 100 g/ kg	DMI, WCW (⇔), ADG (⇑), FCE (⇓)	pH, color, cooking loss, IMF (⇔), WBSF, TBARS (⇓)	Zhao et al. (2018)
Dried grape pomace, 50 g/ kg	N/A	TVC, pseudomonas, (⇑), *Enterobacteriaceae*, Lactic acid bacteria, L*, a*, TBARS (⇔),	Guerra-Rivas et al. (2016)
Destoned exhausted olive cake, 80, 160 and 240 g/ kg	DMI, ADG, FCE (⇔)	Moisture, CP, IMF (⇔)	Kotsampasi et al. (2017)
Grape seed extract, 25 g/kg	N/A	TBARS (⇓)	Jerónimo et al. (2012)
Pomegranate byproduct silage 120 and 240 g/ kg	DMI, ADG, FCE, WCW (⇔)	IMF (⇑) Moisture, protein (⇔)	Kotsampasi et al. (2014)
Dried citrus pulp, 240 and 350 g/kg	N/A	TBARS (⇓), a*, b*, C(⇑)	Inserra et al. (2014)

(⇔) – no difference to the control; (⇑) – increased; (⇓) – decreased.
a*, redness, ADG, average daily gain; b*, yellowness; C, chroma; CP, crude protein; DMI, dry matter intake; FCE, food conversion efficiency; IMF, intramuscular fat; TBARS, thiobarbituric acid reactive substances; TVC, total viable count; WBSF, Warner-Braztler shear force; WCW, warm carcass weight.

2.3.2.1 Potential meat loss reduction strategies

Fruit byproducts are among the richest sources of natural phytochemicals because of the incomplete extraction of phenolics during processing (García-Lomillo and González-SanJosé, 2017). Kasapidou et al. (2015) suggests that utilization of fruit byproducts has positive environmental, economic and social factors in farm animal nutrition. Use of fruit byproducts is also judicious with today's well-informed consumers who are increasingly concerned about the safety of synthetic preservatives in their diets (Papuc et al., 2017). Plant-derived ingredients are widely accepted by consumers as preservatives as they comply with their preference for natural ingredients in meat. Many fruit byproducts have been tested successfully in animal feed and meat preservation as alternative sources of antioxidants and antimicrobials (Table 2.3). According to Kasapidou et al. (2015), the main limitations to the use of fruit byproducts as alternative animal feed ingredients in ruminant nutrition include the presence of anti-quality factors, logistics involved in their processing and the transfer of profits from the feed industry to the animal producer level.

The bio-residues resulting from the wine industry are a good source of bioactive phenolic compounds with antioxidant and antimicrobial effects and can be applied in several industries. The high fiber content of GP makes it a good candidate as an ingredient for ruminant feed through alleviation of feed scarcity, especially in areas where the byproduct is abundant. The bioactivity of fruit byproducts on meat quality and shelf life depends on the extent to which larger oligomers like proanthocyanidins are digested, and/ or their trimeric, dimeric, monomeric units or their metabolites are absorbed (Martinez-Fernandez et al., 2017). Proanthocyanidins with high mean degree of polymerization cannot be easily digested, let alone absorbed (Zhang et al., 2016). A large proportion of proanthocyanidins are associated with dietary fiber, that is, insoluble-bound/ non-extractable polyphenols (Bohn, 2014) and may therefore affect their bioavailability in muscle tissue. López-Andrés et al. (2013) observed that proanthocyanidins from a quebracho were not degraded or

absorbed in the gastrointestinal tract of lambs but still induced antioxidant effects in tissues. This is because microbial fermentation in the reticulo-rumen degrade these non-absorbable oligomers into their respective trimers, dimers and monomers (Martinez-Fernandez et al., 2017), which can thus be absorbed post-ruminally and deposited in tissues enabling them to exert the antioxidative and antimicrobial effects consequently improving meat shelf life. Few studies have, however, been conducted on the digestion and/ or absorption of proanthocyanidins with studies mostly limited to *in vitro* and *in vivo* studies in rats, pigs and humans (Brenes et al., 2016).

2.4 Potential of improving sheep production and product quality using grape pomace

2.4.1 Grape pomace production

Grapes are one of the most widely produced fruit crops in the world and majority are used for wine production, which generates large quantities of solid waste (Brenes et al., 2016). Although GP has been undervalued as a waste, it contains important vitamins, minerals, lipids, proteins, carbohydrates and a complex pool of polyphenolic compounds (Winkler et al., 2015). In the past two decades, scientific interest has grown on the use of GP as a feed for ruminants largely dedicated to investigating its potential as a source of beneficial polyphenolic antioxidants (Tayengwa and Mapiye, 2018). The phytochemical profile of GP supports its use as an interesting source of bioactive compounds and ingredient.

2.4.2 Grape pomace nutrient composition

Grape pomace is the main fraction of the solid wastes, up to 250 g/ kg of the received grapes (García-Lomillo and González-SanJosé, 2017). On average, stalks account for 20 g/kg, seeds 470 g/ kg and skin and pulp 510 g/ kg (Zhang et al., 2017). The various factors involved in the production

of wine influences the nutritional composition of GP. The variability in the nutritional composition (Table 2.4) means that recommendations for its inclusion in ruminant diets can only be done after chemical characterization. The crude protein levels of GP (Table 2.4) are generally above the normal range for maintenance requirements for most low-producing ruminants (60 – 80 g/ kg DM) (National Research Council, 2007) and minimum CP of 80 g/ kg DM required for optimum growth of rumen microbes. The high neutral detergent fiber (NDF) content of between 306 and 626 g/ kg DM (Table 2.4) makes GP a suitable fiber source in ruminant diets. However, the high acid detergent lignin (ADL) levels ranging between 194 and 446 g/ kg DM can be a limitation for its use in ruminant diets, especially for young growing animals because of the possible decrease in digestibility. Therefore, it is important to cautiously select for varieties with low lignin content.

Red GP contains less sugars compared to the white GP because of the fermentation process, which occurs during red wine production (Yu and Ahmedna, 2013). The major lipid contribution in GP are derived from the seeds and constitutes 110 – 190 g/ kg DM oil with a fatty acid (FA) profile rich in PUFA (i.e., 630 – 750 g/ kg DM), monounsaturated FA (140 – 220 g/ kg DM) and low levels of SFA (100 – 150 g/ kg DM) (García-Lomillo and González-SanJosé, 2017; Lutterodt et al., 2011). Linoleic acid is the main FA, which is approximately 70% of total FA (Lutterodt et al., 2011). The seeds contribute non-digestible carbohydrates ranging between 600 and 700 g/ kg DM and contains non-phenolic antioxidants such as tocopherols and β-carotene (Yu and Ahmedna, 2013).

Table 2.4 Chemical composition of grape pomace (g/ kg DM)

Item	Content	References
Dry matter	243 - 952	(Basalan et al., 2011; Baumgärtel et al., 2007; Winkler et al., 2015)
Organic matter	827 - 971	(Baumgärtel et al., 2007; Molina-Alcaide et al., 2008; Ribeiro et al., 2015)
Crude protein	53.2 - 155	(Basalan et al., 2011; Baumgärtel et al., 2007; Ribeiro et al., 2015)
Fat	47.7 - 135	(Ishida et al., 2015; Llobera and Cañellas, 2007; Molina-Alcaide et al., 2008)
Neutral detergent fiber	306 - 626	(Alipour and Rouzbehan, 2007; Baumgärtel et al., 2007; Molina-Alcaide et al., 2008)
Acid detergent fiber	294 - 526	(Basalan et al., 2011; Molina-Alcaide et al., 2008; Zalikarenab et al., 2007)
Acid detergent lignin	194 - 446	(Alipour and Rouzbehan, 2007; Molina-Alcaide et al., 2008; Zalikarenab et al., 2007)
Acid detergent insoluble nitrogen	0.20 - 0.46	(Molina-Alcaide et al., 2008)
Soluble sugars	18.7 - 276	(Baumgärtel et al., 2007)
Non-fiber carbohydrates	263 - 443	(Basalan et al., 2011; Ishida et al., 2015)
Metabolizable energy[1]	6.85 - 6.86	(Basalan et al., 2011)
Calcium[2]	113 - 3800	(Eleonora et al., 2014; Ribeiro et al., 2015; Zhao et al., 2018)
Phosphorus[2]	182 - 4200	(Eleonora et al., 2014; Ribeiro et al., 2015; Zhao et al., 2018)
Magnesium[2]	1300	(Eleonora et al., 2014)
Manganese[2]	19.2 - 656	(Ribeiro et al., 2015)
Potassium[2]	1097 - 1956	(Ribeiro et al., 2015)
Iron[2]	6.91 - 49.9	(Ribeiro et al., 2015)
Total phenolic content	25.3 – 87.0[a]	(González-Centeno et al., 2013; Winkler et al., 2015)
	19.6 – 70.5[b]	(Abarghuei et al., 2010; Zalikarenab et al., 2007)
Total tannins	15.4 – 52.0[b]	(Abarghuei et al., 2010; Eleonora et al., 2014; Zalikarenab et al., 2007)
Proanthocyanidins	50.8 – 92.1[c]	(Abarghuei et al., 2010; González-Centeno et al., 2013)
	90.5 – 223[d]	(Llobera and Cañellas, 2007; Molina-Alcaide et al., 2008)
	79[e]	(Abarghuei et al., 2010)
	21 – 36[f]	(Baumgärtel et al., 2007)

[1] MJ/ kg DM.
[2] mg/ kg DM.
[a] g/ kg gallic acid equivalent.
[b] g/ kg tannic acid equivalent.
[c] g/ kg tannins.
[d] g/ kg purified condensed tannins.
[e] g/ kg tannins.
[f] g/ kg catechin equivalent

2.4.3 Profile and biological activity of phenolic compounds in grape pomace

Flavonoids, especially the subgroup proanthocyanidins, are most abundant in skins and seeds, followed by anthocyanins, mostly located in the skin (Fig. 2.1) (Beres et al., 2017; Fontana et al., 2013). The ratio of seeds, skins and stalks are a major contributory factor to the variation in GP phenolic content. Of the total flavanols of grapes, seeds have greater flavanols content (560 – 650 g/

kg DM) relative to skins (140 – 210 g/ kg DM) (García-Lomillo and González-SanJosé, 2017). It should be noted that the bioactive profile and content are strongly influenced by genetic factors, environmental conditions and the wine-making technology (Garrido and Borges, 2013; Shi et al., 2003).

Proanthocyanidins are oligomeric and polymeric-linked flavonoid units synthesized in the flavonoid pathway. The biological activity of proanthocyanidins is determined by the monomer type, stereochemistry of the heterocyclic C – ring and the mean degree of polymerization (Garrido and Borges, 2013). The following characteristics are known to increase the antioxidative capacity of phenolic compounds, including those found in GP: the presence of a 3′,4′-dihydroxy structure in the B ring. Additionally, the presence of a 2,3-double bond in conjunction with the 4-*oxo* group in the heterocycle, allowing for conjugation between the A and B rings. Lastly, the presence of 3- and 5-hydroxyl groups in the A ring together with a 4-*oxo* function in the A and C rings (Fig. 2.2) (Apak et al., 2016; Garrido and Borges, 2013). It has been reported that the antioxidant power of proanthocyanidins is 20 times greater than α-tocopherol and 50 times greater than ascorbic acid (Shi et al., 2003).

Available research has shown that the antimicrobial properties of polyphenols are associated with their ability to interact with either cell wall or the cell membranes of bacteria through hydrogen – bonding and hydrophobic effects and lipophilic forces, in addition to covalent bond formation (Papuc et al., 2017). According to Scalbert (1991), the chelation of polyphenols with some metal ions is vital for the survival of bacteria (e.g., iron) and is the property responsible for their antibacterial activity. Due to the significance of proanthocyanidins in animal nutrition, the following section of the literature review will basically focus on this phenolic group.

Although the concentration of proanthocyanidins in GP ranges from as low as 21 g/ kg DM to as high as 223 g/ kg DM (Table 2.4), the final concentration in ruminant diet is usually lower than 50 g/

kg DM (Abarghuei et al., 2010; Baumgärtel et al., 2007; Zalikarenab et al., 2007), a level considered beneficial for ruminant nutrition. These levels are usually achieved due to the dilution effects with other non-proanthocyanidin containing ingredients in the diet (Waghorn, 2008). However, concentration of proanthocyanidin in the diet is not the only factor to consider in ruminant nutrition, especially with regards to nutrient digestibility but other factors such as the structure and source of this phenolic group, as well as the lignin content and composition of the basal diet need to be considered as well (Jayanegara and Palupi, 2010). Mueller-Harvey et al. (2007) demonstrated that ruminants in the tropics can tolerate proanthocyanidin concentration above 80 g/ kg DM. From a nutritional point of view, with regards to the protein and fiber contents, along with some antioxidant phenolic compounds, dietary GP can be used as a natural preservative of meat. The benefits of proanthocyanidins on animal health, growth and meat quality can only be realized when the composition, structure and biological function are well-defined.

The content and bioactive compounds of GP is also influenced by the ratio of GP components (García-Lomillo and González-SanJosé, 2017; Garrido and Borges, 2013). Seed proanthocyanidin are composed of shorter polymers of similar amounts of catechin and epicatechin subunits, while in the skin the polymers tend to be much longer and comprised mainly of epicatechin subunits (Downey et al., 2006). One more example is that anthocyanins are only found in the skin of the grape, while the flavonoids occur both in the skin and seeds (Garrido and Borges, 2013). If pomace contains more seeds, it will subsequently have greater PUFA content because the majority of the fatty acids are concentrated in the seeds more than the skins (García-Lomillo and González-SanJosé, 2017; Lutterodt et al., 2011). The preceding factors are just a few examples which influence the bioactive profile of GP. However, preservation of GP can also influence phenolic content and profile. Preservation method also plays a crucial role in the bioactive profile of GP. During the preservation ruminant feed resources are either ensiled or dehydrated.

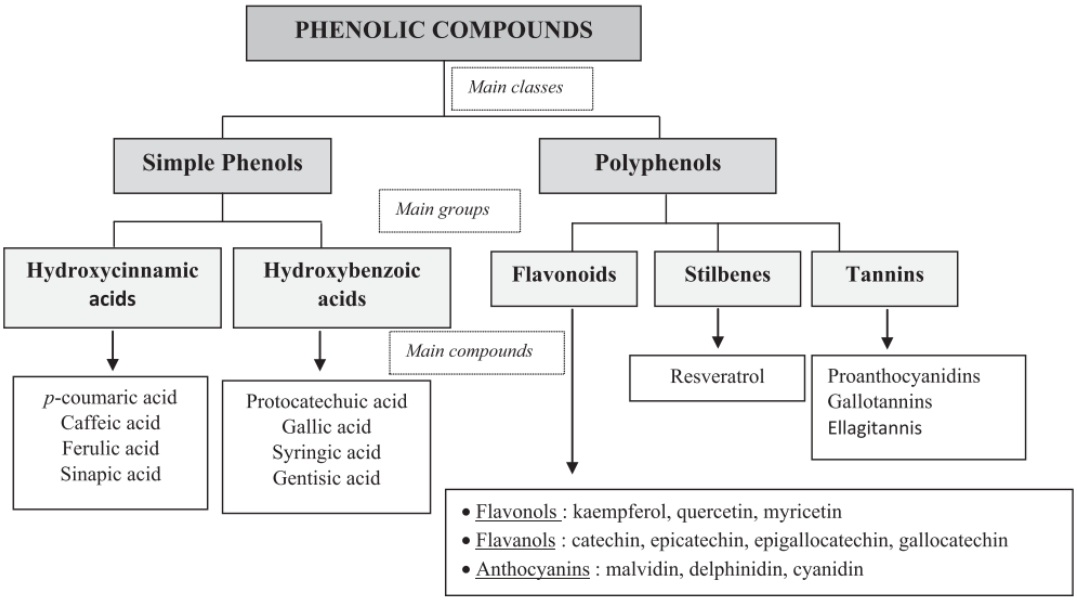


Fig. 2.1 Main phenolic compounds found in grape pomace. Source: (Beres et al., 2017).

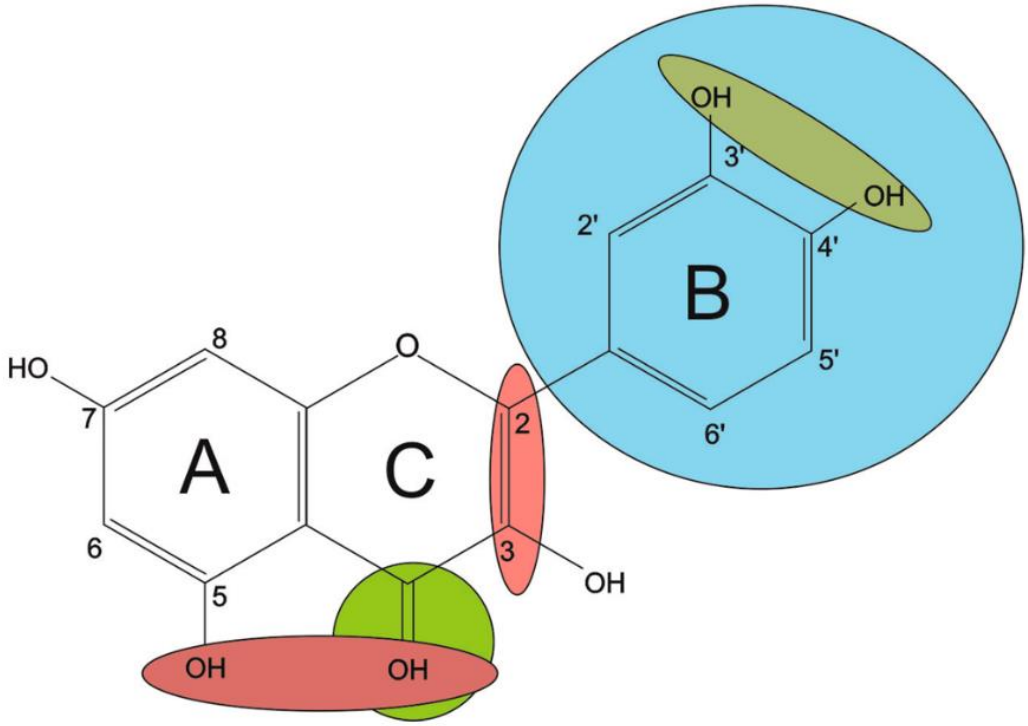


Fig. 2.2 Characteristic functional groups having a key role in the high antioxidant capacity of a flavonoid. Source: (Apak et al., 2016).

2.4.4 Effects of dehydration of grape pomace on nutrient and bioactive compounds

Fresh pomace is a highly perishable product owing to its high moisture (500 – 720 g/ kg DM) and sugar contents (18.7 – 276 g/ kg DM) (Baumgärtel et al., 2007; García-Lomillo and González-SanJosé, 2017). Owing to the short harvesting season and the large quantities produced during this period, GP has to be preserved to avoid spoilage and for long term use. This is particularly important because of the costs and logistics involved when transporting GP in its original wet form (Goula et al., 2016). Depending on the type of drying method, significant losses of phenolics and a decrease in the radical scavenging activity may occur (Tseng and Zhao, 2012). There is limited information on the effects of dehydration methods on the concentration of proanthocyanidins in GP. The reduction of moisture content through drying is employed to slow down or inhibit microbial contamination, therefore increase its shelf life, at the same time reduce storage space required, over and above weight savings (Sui et al., 2014). Drying also assists in the release of phenolics through the creation of large cavities and intercellular spaces as breakage and destruction of cell walls occurs during this process (Drosou et al., 2015). However, phenolic compounds are sensitive to environmental factors such as high temperatures and oxidizing agents (Larrauri et al., 1997; Tseng and Zhao, 2012). In dehydrated GP, non-enzymatic oxidation process is more prevalent than the enzymatic process due to the low water content, a crucial medium for enzyme activity (Domínguez et al., 2017). Investigating the effects of different dehydration methods vis-à-vis the concentration of GP proanthocyanidins and their effect on bioavailability and utilization in ruminants is, therefore, important.

2.4.4.1 Freeze drying

Freeze-drying is regarded as one of the best methods of preserving bioactive compounds (Çoklar and Akbulut, 2017; Tseng and Zhao, 2012) because it uses low temperatures and pressure conditions, which are usually less degradative, especially to phenolic compounds (García-Lomillo and González-

SanJosé, 2017). Çoklar and Akbulut (2017) and De Torres et al. (2015) observed no significant differences in the quantity of phenolic compounds of grapes and their byproducts after thermal treatments, respectively. In addition, Tseng and Zhao (2012) reported that freeze-dried GP retained the highest bioactivity compared to oven (40 °C) or air-dried (25 °C) samples. Generally, freeze drying conditions are mild and therefore enhance the stability and preservation of heat labile bioactive compounds (Çoklar and Akbulut, 2017; García-Lomillo and González-SanJosé, 2017). In contrast, Larrauri et al. (1997) and Tseng and Zhao (2012) reported low stability of phenolics for freeze-dried products during storage. This is attributed to the high porosity created during the freeze-drying process and often leads to increased air contact and susceptibility to oxidation (García-Lomillo and González-SanJosé, 2017). The porosity and subsequent processes may result in low content of phenolics in freeze-dried products over time. Furthermore, freeze-drying is estimated to be 4 – 8 times more expensive than thermal drying methods (García-Lomillo and González-SanJosé, 2017). Therefore, freeze-drying would have no practical application at all due to cost implications. Thermal drying techniques such as oven and sun are more feasible for preservation of large quantities of pomace produced during the harvesting season, particularly for low-input ruminant livestock production systems.

2.4.4.2 Oven drying

Processing techniques that employ the use of thermal treatments have been associated with increasing the extractability and bioavailability of some polyphenols, but at the same time may lead to destruction of some heat-sensitive polyphenols (Khanal et al., 2010; Larrauri et al., 1997). High temperatures result in chemical degradation, isomerization and/ or polymerization of bioactive compounds and subsequently, loss of their bioactivity (Larrauri et al., 1998; Yu and Ahmedna, 2013). Sui et al. (2014) noted that as the oven temperature increases from 60 to 90 °C, proanthocyanidin content declined by 4%. Khanal et al. (2010), however, reported that temperatures of 40 °C for 72

hours had no detrimental effect on the proanthocyanidins profile. Conversely, greater reductions (43%) in GP proanthocyanidin content were observed by Planinić et al. (2015) at 80 °C regardless of drying time. Larrauri et al. (1997) did not report any difference in proanthocyanidin content between freeze- and oven-dried (60 °C) red GP but observed a decline of 11.1 and 16.6% at 100 and 140 °C, respectively. Interestingly, controversial findings were reported by Kim et al. (2006) who observed that thermally processed whole and powdered grape seeds showed a better *in vitro* antioxidant activity compared to untreated grape seeds. The preceding results show no consensus regarding the optimum thermal drying temperature. Nonetheless, from the literature reviewed interrogated above, it would be advisable to dry GP at temperatures between 20 and 60 °C so as to and still maintain some levels of bioactivity (Drosou et al., 2015; Larrauri et al., 1997; Sui et al., 2014) or alternatively use sun-drying which tends to make use of lower temperature regimes.

2.4.4.3 Sun drying

Sun drying is one of the most widely used methods to produce dried foods and agricultural products due to the low investment and operating cost (Çoklar and Akbulut, 2017; Sui et al., 2014), notwithstanding the sensitivity of polyphenols to ultraviolet radiation. Nonetheless, the lower drying temperatures involved, and negligible energy costs still favor the use of this technique, more so for ruminant feeds such as GP because of its seasonality and the large quantities generated over a short period of time (Beres et al., 2017). Sun drying has been used for preservation of GP as a source of fiber and antioxidants in rabbits (Guemour et al., 2010) and sheep (Abarghuei et al., 2010). There is limited data on the effect of sun drying on proanthocyanidin content with most of the studies (examples) focusing on other fruit component rather than the GP.

Bagheripour et al. (2008) reported an increase in proanthocyanidin levels after sun drying pistachio byproducts (13.3 g/ kg DM) relative to freeze-dried (9.14 g/ kg). This increase could be

ascribed to the polymerization of flavan-3-ols with complex macromolecules or the loss of hexoses as a result of respiration, which may have led to an increase in the concentration of proanthocyanidins (McDonald et al., 2011). Alternatively, sun drying may produce oxidation products through microbial enzymes or because of the presence of atmospheric oxygen (García-Lomillo and González-SanJosé, 2017). In contrast, a study by Makkar and Singh (1991) showed no change in the concentration of proanthocyanidins for sun-dried oak leaves for 48 hours. However, sun drying led to a reduction in proanthocyanidins in cassava and leucaena leaves, which had a higher water content (650 vs. 400 g/ kg DM) than the oak leaves (Makkar and Singh, 1991). Therefore, sun drying could be an efficient and cheaper method for preservation of GP and possibly reduce proanthocyanidins for incorporation in ruminant diets.

2.5 Effect of feeding grape pomace on animal production

Researchers have investigated the impact of GP polyphenolic compounds both *in vitro* and *in vivo* on nutrient digestibility (Baumgärtel et al., 2007; Winkler et al., 2015), ruminal parameters (Abarghuei et al., 2010; Ishida et al., 2015), methane emission (Moate et al., 2014), nitrogen (N) excretion (Greenwood et al., 2012), and growth performance, meat quality and shelf life (Guerra-Rivas et al., 2016; Zhao et al., 2018). Given their high phenolic contents and the related antioxidant and antimicrobial properties, the inclusion of GP in feed rations would reduce the use of expensive synthetics and consequently enhance the oxidative stability of meat (Brenes et al., 2016; Guerra-Rivas et al., 2016; Manso et al., 2016), thus helping to meet consumer demand for healthier meat products.

2.5.1 Effect of feeding grape pomace on nutrient digestibility

Grape pomace is a promising ruminant feed ingredient because it is an abundant and less expensive source of polyphenols than commercial fiber sources, such as lucerne (Winkler et al., 2015). Nonetheless, it has been reported that GP proanthocyanidins alter rumen fermentation and

reduce microbial protein yield (Abarghuei et al., 2010; Ishida et al., 2015). Furthermore, the high fiber content in GP is associated with reduced nutrient digestibility (Baumgärtel et al., 2007).

Abarghuei et al. (2010) fed GP as a supplement in lamb diets at 762 g/ kg DM and observed reductions in organic matter and NDF digestibility, urinary N, N retention, ruminal ammonia concentration, microbial protein production, and cellulolytic and proteolytic bacterial populations. Ishida et al. (2015) reported similar findings with the exception of N retention, despite the increased fecal N losses in GP based diets. Lignin and proanthocyanidins usually form cross linkages with polysaccharides, subsequently acting as physical barriers to microbial enzymes reaching their target polysaccharides (Alipour and Rouzbehan, 2007; Moore and Jung, 2001). Protein–proanthocyanidin complexes formed in the rumen slow the rate of fermentation, mainly through the inhibition of the activities of proteolytic microbes (Frutos et al., 2004). This subsequently prevents the degradation of dietary CP and the decline in N digestibility and ruminal ammonia–nitrogen production (Frutos et al., 2004; Patra and Saxena, 2009). The higher the lignin content, the less NDF intake by the animal as the digesta is retained longer within the rumen (Mertens, 1994). Furthermore, the presence of proanthocyanidins could have an additive effect on decreased polysaccharide intake and digestibility.

Grape pomace fed to dairy cows reduced methane and urea production (Moate et al., 2014). High levels of urea and methane gas are produced by ruminants, especially those on high protein diets (Moate et al., 2014). Although, the exact mechanisms are not fully understood, it has been hypothesized that proanthocyanidins selectively bind to methane and ammonia-producing bacterial enzymes (Patra and Saxena, 2010). Binding of polysaccharides and proteins would also reduce their fermentation, which could lead to decrease in ammonia and methane production (Patra and Saxena, 2010). Thus, feeding GP subsequently reduces ammonia production leading to lower plasma urea N and less urinary N excretion (Foiklang et al., 2016; Moate et al., 2014). Proanthocyanidins inhibit growth of slime producing ruminal bacteria and precipitate soluble protein products which have been

implicated in the reduction of frothy bloat in grazing cattle (Patra and Saxena, 2010). Feeding diets with moderate levels (20 – 50 g/ kg DM) of proanthocyanidins simultaneously decreases N losses, methane production and frothy bloat, thereby improving the productivity and health of animals.

2.5.2 Effect of feeding grape pomace on growth performance, carcass traits and meat quality

In the Mediterranean countries, GP has been used as an alternative feed source for sheep when conventional feed supplies are scarce (Manso et al. 2016). Although, GP is low in highly fermentable carbohydrate content, it can be used as an ingredient for ruminant diets when fed close to maintenance levels (Baumgärtel et al., 2007). Its fiber and CP contents are comparable to mature lucerne hay which is a common forage source for growing lambs (Calderón-Cortés et al., 2018). Its high phenolic content may have negative associative effects with other components of the diet that could affect the feeding value (Calderón-Cortés et al., 2018). However, when fed with other ingredients of low tannin content, GP can be incorporated in ruminant diets up to a maximum level of 300 g/ kg DM (Zepf and Jin, 2013).

Zhao et al. (2018) reported that inclusion of the GP at 100 g/ kg DM increased body weight and average daily gain, albeit a reduction in the feed to gain ratio. No effects on carcass traits and meat quality were observed but collagen content decreased lowering the Warner–Bratzler shear force (Zhao et al., 2018). Tanniferous diets negatively affect the rate of fiber digestion, thus slowing down the clearance of feed residues from the rumen which may necessitate more rumination and, consequently reduce voluntary feed intake, especially at higher inclusion levels (Waghorn, 2008). In addition, proanthocyanidins produce astringent sensations and a bitter taste which at higher levels may induce aversion and also interact with salivary glycoproteins, modulating their post-ingestive effects (Frutos et al., 2004; Makkar, 2003). Increasing GP beyond 100 g GP / kg of diet DM decreases

the energy value because of the negative associative effects of proanthocyanidins which are known to form complexes with macromolecules, particularly proteins and to a lesser extent lipids, polysaccharides and minerals (Calderón-Cortés et al., 2018). Therefore, reduction of voluntary feed intake and digestibility leads to poor growth rates of animals. Proanthocyanidin have antimicrobial activity on pathogenic gram-negative bacteria, as well as strong antioxidant activity therefore their intake in diets would be beneficial to the overall growth and health of the animal.

Although Zhao et al. (2018) did not observe differences in carcass weight in lambs supplemented at 100 g GP / kg of diet DM, Kafantaris et al. (2018) reported higher cold carcass weights for lambs fed 90 g GP / kg of diet DM compared to the control. Apart from the GP content, differences between these two studies could have emanated from the basal diet. Feed containing moderate levels of proanthocyanidins also improves meat lightness possibly because of reduced production of B12 vitamin by ruminal microorganisms, as found *in vitro* resulting in a reduced production of hemoglobin (Priolo and Vasta, 2007). This is contrary to findings by Jerónimo et al. (2012) who did not observe an improvement in meat lightness after dietary supplementation of lambs with grape seed extract.

2.5.3 Effect of feeding grape pomace on meat shelf stability

Biochemical reactions in meat involving macromolecules (myoglobin, lipid and protein) contribute to rancidity, discoloration, loss of nutritional quality including essential amino acids and protein digestibility, and loss of protein functionality (Cunha et al., 2018; Sun and Holley, 2012). The nutrient-rich matrix of meat offers excellent conditions favorable for the growth of either spoilage or pathogenic microorganisms (Sun and Holley, 2012). Both oxidation and microbial contamination not only reduce the shelf life of meat but also in quality deterioration making it undesirable or unsuitable for sale or consumption (Li and Liu, 2012; Papuc et al., 2017). Chemically, myoglobin, lipid and protein oxidation in meat are interrelated, as one can exacerbate the occurrence of the other (Cunha

et al., 2018; Li and Liu, 2012). Synthetic antioxidant and antimicrobials have been used for a long time for meat preservation (Li and Liu, 2012; Papuc et al., 2017). However, the modern consumer is more concerned about their diet and have a greater preference for natural preservatives. Thus, researchers are currently in search for alternative natural preservatives which are as effective as the current synthetics or better, but without compromising meat quality.

Research has focused on fruit byproducts such as GP because of the high phenolic content, especially the polymeric proanthocyanidin which has antioxidant and antimicrobial properties because the high degree of active hydroxyl sites (Apak et al., 2016; Li and Liu, 2012; Sun and Holley, 2012). Presence of the 3′,4′-dihydroxy substituents in the C ring and conjugation between the A and B rings, have highly effective radical scavenging structures and also augment the activity of endogenous antioxidants (Apak et al., 2016). Besides acting as proton donors and thus terminating oxidation, proanthocyanidins have been shown to delay endogenous antioxidants from degrading (Chaijan, 2008). Alternatively, proanthocyanidins can chelate transition metals (e.g., iron and copper) which act as catalysts in lipid oxidation in the reaction known as the Haber-Weiss reaction (Apak et al., 2016; Li and Liu, 2012).

Few studies that investigated the effect of dietary grape byproducts on the shelf stability of lamb meat showed a reduction in lipid oxidation during storage (Guerra-Rivas et al., 2016; Jerónimo et al., 2012). Similarly, Kafantaris et al. (2017) observed decreased oxidative stress-induced damage to lipids and proteins in lambs fed silage-based diets containing 90 g GP / kg of diet DM. The addition of natural antioxidants to feed not only can improve the oxidative stability and organoleptic properties of meat but they also can enhance the nutritional value and the health benefit of meat product.

2.6 Summary

There is, therefore, a knowledge gap for optimum utilization of this locally available and cheap source of polyphenols in feedlot feeding of lambs, especially in the Cape Winelands regions of South

Africa where GP is found in abundance. Dietary supplementation of GP has the potential to simultaneously improve growth performance and nutrient utilization when added at low to moderate concentration. At the same time, GP could maintain desirable meat redness, reduce microbial growth and oxidation of lipid and proteins in meat because of moderate levels of proanthocyanidins. Overall, thermally-dried GP can be a useful strategy for improving ruminant animal growth, health, meat quality because of its antioxidant and antimicrobial properties. Indirect environmental benefits can be achieved through the reduction of methane emission and nitrogen excretion. Thus, potentially solving the current problems associated with GP disposal.

2.7 References

Abarghuei, M.J., Rouzbehan, Y., Alipour, D., 2010. The influence of the grape pomace on the ruminal parameters of sheep. Livest. Sci. 132, 73–79.

AHDB (Agriculture and Horticulture Development Board), 2016. World sheep meat market to 2025 World sheep meat. http://www.meat-ims.org/wp-content/uploads/2016/01/World-sheep-meat-market-to-2025. (accessed 10.26.16).

AHDB (Agriculture and Horticulture Development Board), 2015. UK Yearbook - Sheep. http://beefandlamb.ahdb.org.uk/wp/wp-content/uploads/2015/07/UK-Yearbook-2015-Sheep-200715.pdf (accessed 10.26.16)

Alipour, D., Rouzbehan, Y., 2007. Effects of ensiling grape pomace and addition of polyethylene glycol on in vitro gas production and microbial biomass yield. Anim. Feed Sci. Technol. 137, 138–149.

Apak, R., Özyürek, M., Güçlü, K., Çapanoɨlu, E., 2016. Antioxidant activity/capacity measurement. 1. Classification, physicochemical principles, mechanisms, and electron transfer (ET)-based assays. J. Agric. Food Chem. 64, 997–1027.

Bagheripour, E., Rouzbehan, Y., Alipour, D., 2008. Effects of ensiling, air-drying and addition of

polyethylene glycol on in vitro gas production of pistachio byproducts. Anim. Feed Sci. Technol. 146, 327–336.

Basalan, M., Gungor, T., Owens, F.N., Yalcinkaya, I., 2011. Nutrient content and in vitro digestibility of Turkish grape pomaces. Anim. Feed Sci. Technol. 169, 194–198.

Baumgärtel, T., Kluth, H., Epperlein, K., Rodehutscord, M., 2007. A note on digestibility and energy value for sheep of different grape pomace. Small Rumin. Res. 67, 302–306.

Ben Salem, H., 2010. Nutritional management to improve sheep and goat performances in semiarid regions. Rev. Bras. Zootec. 39, 337–347.

Beres, C., Costa, G.N.S., Cabezudo, I., da Silva-James, N.K., Teles, A.S.C., Cruz, A.P.G., Mellinger-Silva, C., Tonon, R.V., Cabral, L.M.C., Freitas, S.P., 2017. Towards integral utilization of grape pomace from winemaking process: A review. Waste Manag. 68, 581–594.

Bohn, T., 2014. Dietary factors affecting polyphenol bioavailability. Nutr. Rev. 72, 429–452.

Brand, T.S., Genis, M.P., Hoffman, L.C., van de Vyver, W.F.J., Swart, R., Jordaan, G.F., 2013. The effect of dietary energy and the inclusion of a β-adrenergic agonist in the diet on the meat quality of feedlot lambs. S. Afr. J. Anim. Sci. 43, S140–S145.

Brenes, A., Viveros, A., Chamorro, S., Arija, I., 2016. Use of polyphenol-rich grape byproducts in monogastric nutrition. A review. Anim. Feed Sci. Technol. 211, 1–17.

Buys, E.M., Nortjé, G.L., Jooste, P.J., Von Holy, A., 2000. Combined effect of modified atmosphere bulk packaging, dietary vitamin E supplementation and microbiological contamination on colour stability of Musculus gluteus medius. Meat Sci. 55, 403–411.

Calderón-Cortés, J.F., González-Vizcarra, V.M., Pétriz-Celaya, Y., Pujol, L.C., Barreras, A., Plascencia, A., 2018. Energy value of unfermented dried grape pomace as substitute of alfalfa hay in diets for growing lambs. Aust. J. Vet. Sci. 63, 59–63.

Caparra, P., Foti, F., Scerra, M., Sinatra, M.C., Scerra, V., 2007. Solar-dried citrus pulp as an

alternative energy source in lamb diets: Effects on growth and carcass and meat quality. Small Rumin. Res. 68, 303–311.

Chaijan, M., 2008. Review: Lipid and myoglobin oxidations in muscle foods. Songklanakarin J. Sci. Technol. 30, 47–53.

Cloete, S.W., Olivier, J.J., 2010. South African sheep and wool Industry, in: International Sheep and Wool Handbook. pp. 95–112.

Çoklar, H., Akbulut, M., 2017. Effect of sun, oven and freeze-drying on anthocyanins, phenolic compounds and antioxidant activity of Black grape (Ekşikara) (Vitis vinifera L.). South African J. Enol. Vitic. 38, 264–272.

Cunha, L.C.M., Monteiro, M.L.G., Lorenzo, J.M., Munekata, P.E.S., Muchenje, V., de Carvalho, F.A.L., Conte-Junior, C.A., 2018. Natural antioxidants in processing and storage stability of sheep and goat meat products. Food Res. Int. 111, 379–390.

DAFF (Department of Agriculture, Forestry and Fisheries), 2018. Statistics and Economic Publications and Reports- Livestock numbers from 1996 to current. https://www.daff.gov.za/daffweb3/Home/Crop-Estimates/Statistical-Information/Livestock. (accessed 10.13.18).

de Torres, C., Schumacher, R., Alañón, M.E., Pérez-Coello, M.S., Díaz-Maroto, M.C., 2015. Freeze-dried grape skins byproducts to enhance the quality of white wines from neutral grape varieties. Food Res. Int. 69, 97–105.

Díaz, M.T., Velasco, S., Cañeque, V., Lauzurica, S., Ruiz De Huidobro, F., Pérez, C., González, J., Manzanares, C., 2002. Use of concentrate or pasture for fattening lambs and its effect on carcass and meat quality. Small Rumin. Res. 43, 257–268.

Domínguez, J., Sanchez-Hernandez, J.C., Lores, M., 2017. Vermicomposting of winemaking byproducts, in: Galanakis, C. (Ed.), Handbook of Grape Processing Byproducts. Academic

Press, London UK, pp. 55–78.

Downey, M.O., Dokoozlian, N.K., Krstic, M.P., 2006. Cultural practice and environmental impacts on the flavonoid composition of grapes and wine: A review of recent research. Am. J. Enol. Vitic. 57, 257–268.

Drosou, C., Kyriakopoulou, K., Bimpilas, A., Tsimogiannis, D., Krokida, M., 2015. A comparative study on different extraction techniques to recover red grape pomace polyphenols from vinification byproducts. Ind. Crops Prod. 75, 141–149.

Eleonora, N., Alina, D., Valeria, C., 2014. Grape pomace in sheep and dairy cows feeding. J. Hortic. For. Biotechnol. 18, 146–150.

FAO (Food and Agriculture Organization), 2013. Food Outlook: Biannual Report on Global Food Markets.

FAOSTAT (Food and Agriculture Organization Corporate Statistical Database), 2016. Live animals. www.fao.org/faostat/en/#data/QA (accessed 1.21.17).

Foiklang, S., Wanapat, M., Norrapoke, T., 2016. Effect of grape pomace powder, mangosteen peel powder and monensin on nutrient digestibility, rumen fermentation, nitrogen balance and microbial protein synthesis in dairy steers. Asian-Australasian J. Anim. Sci. 29, 1416–1423.

Fontana, A.R., Antoniolli, A., Bottini, R., 2013. Grape pomace as a sustainable source of bioactive compounds: Extraction, characterization, and biotechnological applications of phenolics. J. Agric. Food Chem. 61, 8987–9003.

Frutos, P., Hervás, G., Mantecón, A.R., 2004. Tannins and ruminant nutrition. Spanish J. Agric. Res. 2, 191–202.

García-Lomillo, J., González-SanJosé, M.L.M.L., 2017. Applications of wine pomace in the food industry: Approaches and functions. Compr. Rev. Food Sci. Food Saf. 16, 3–22.

Garrido, J., Borges, F., 2013. Wine and grape polyphenols - A chemical perspective. Food Res. Int.

54, 1844–1858.

González-Centeno, M.R., Jourdes, M., Femenia, A., Simal, S., Rosselló, C., Teissedre, P.L., 2013. Characterization of polyphenols and antioxidant potential of white grape pomace byproducts (*Vitis vinifera* L.). J. Agric. Food Chem. 61, 11579–11587.

Goula, A.M., Thymiatis, K., Kaderides, K., 2016. Valorization of grape pomace: Drying behavior and ultrasound extraction of phenolics. Food Bioprod. Process. 100, 132–144.

Greenwood, S.L., Edwards, G.R., Harrison, R., 2012. Supplementing grape marc to cows fed a pasture-based diet as a method to alter nitrogen partitioning and excretion. J. Dairy Sci. 95, 755–758.

Guemour, D., Bannelier, C., Dellal, A., Gidenne, T., 2010. Nutritive value of sun-dried grape pomace, incorporated at a low level in complete feed for the rabbit bred undeŕ magrebian conditions. World Rabbit Sci. 18, 17–25.

Guerra-Rivas, C., Vieira, C., Rubio, B., Martínez, B., Gallardo, B., Mantecón, A.R., Lavín, P., Manso, T., 2016. Effects of grape pomace in growing lamb diets compared with vitamin E and grape seed extract on meat shelf life. Meat Sci. 116, 221–229.

Inserra, L., Priolo, A., Biondi, L., Lanza, M., Bognanno, M., Gravador, R., Luciano, G., 2014. Dietary citrus pulp reduces lipid oxidation in lamb meat. Meat Sci. 96, 1489–1493.

Ishida, K., Kishi, Y., Oishi, K., Hirooka, H., Kumagai, H., 2015. Effects of feeding polyphenol-rich winery wastes on digestibility, nitrogen utilization, ruminal fermentation, antioxidant status and oxidative stress in wethers. Anim. Sci. J. 86, 260–269.

Jayanegara, A., Palupi, E., 2010. Condensed tannin effects on nitrogen digestion in ruminants: A meta-analysis from in vitro and in vivo studies. Media Peternak. 33, 176–181.

Jerónimo, E., Alfaia, C.M.M., Alves, S.P., Dentinho, M.T.P., Prates, J.A.M., Vasta, V., Santos-Silva, J., Bessa, R.J.B., 2012. Effect of dietary grape seed extract and Cistus ladanifer L. in

combination with vegetable oil supplementation on lamb meat quality. Meat Sci. 92, 841–847.

Jones, K.G., 2004. Trends in the US sheep industry, Agriculture Information Bulletin.

Kafantaris, I., Kotsampasi, B., Christodoulou, V., Kokka, E., Kouka, P., Terzopoulou, Z., Gerasopoulos, K., Stagos, D., Mitsagga, C., Giavasis, I., Makri, S., Petrotos, K., Kouretas, D., 2017. Grape pomace improves antioxidant capacity and faecal microflora of lambs. J. Anim. Physiol. Anim. Nutr. (Berl). 101, e108–e121.

Kafantaris, I., Kotsampasi, B., Christodoulou, V., Makri, S., Stagos, D., Gerasopoulos, K., Petrotos, K., Goulas, P., Kouretas, D., 2018. Effects of dietary grape pomace supplementation on performance, carcass traits and meat quality of lambs. In Vivo (Brooklyn). 32, 807–812.

Kasapidou, E., Sossidou, E., Mitlianga, P., 2015. Fruit and vegetable co-products as functional feed ingredients in farm animal nutrition for improved product quality. Agriculture 5, 1020–1034.

Khanal, R.C., Howard, L.R., Prior, R.L., 2010. Effect of heating on the stability of grape and blueberry pomace procyanidins and total anthocyanins. Food Res. Int. 43, 1464–1469.

Kim, S.Y., Jeong, S.M., Park, W.P., Nam, K.C., Ahn, D.U., Lee, S.C., 2006. Effect of heating conditions of grape seeds on the antioxidant activity of grape seed extracts. Food Chem. 97, 472–479.

Kotsampasi, B., Christodoulou, V., Zotos, A., Liakopoulou-Kyriakides, M., Goulas, P., Petrotos, K., Natas, P., Bampidis, V.A., 2014. Effects of dietary pomegranate byproduct silage supplementation on performance, carcass characteristics and meat quality of growing lambs. Anim. Feed Sci. Technol. 197, 92–102.

Kotsampasi, Bampidis, V.A., Tsiaousi, A., Christodoulou, C., Petrotos, K., Amvrosiadis, I., Fragioudakis, N., Christodoulou, V., 2017. Effects of dietary partly destoned exhausted olive cake supplementation on performance, carcass characteristics and meat quality of growing

lambs. Small Rumin. Res. 156, 33–41.

Larrauri, J.A., Rupérez, P., Saura-Calixto, F., 1997. Effect of drying temperature on the stability of polyphenols and antioxidant activity of red grape pomace peels. J. Agric. Food Chem. 45, 1390–1393.

Larrauri, J.A., Sánchez-Moreno, C., Saura-Calixto, F., 1998. Effect of temperature on the free radical scavenging capacity of extracts from red and white grape pomace Peels. J. Agric. Food Chem. 46, 2694–2697.

Li, Y., Liu, S., 2012. Reducing lipid peroxidation for improving colour stability of beef and lamb: On-farm considerations. J. Sci. Food Agric. 92, 719–726.

Llobera, A., Cañellas, J., 2007. Dietary fibre content and antioxidant activity of Manto Negro red grape (*Vitis vinifera*): pomace and stem. Food Chem. 101, 659–666.

López-Andrés, P., Luciano, G., Vasta, V., Gibson, T.M., Biondi, L., Priolo, A., Mueller-Harvey, I., 2013. Dietary quebracho tannins are not absorbed, but increase the antioxidant capacity of liver and plasma in sheep. Br. J. Nutr. 110, 632–639.

Lorenzo, J.M., Gómez, M., 2012. Shelf life of fresh foal meat under MAP, overwrap and vacuum packaging conditions. Meat Sci. 92, 610–618.

Luciano, G., Monahan, F.J., Vasta, V., Biondi, L., Lanza, M., Priolo, A., 2009. Dietary tannins improve lamb meat colour stability. Meat Sci. 81, 120–125.

Luciano, G., Pauselli, M., Servili, M., Mourvaki, E., Serra, A., Monahan, F.J., Lanza, M., Priolo, A., Zinnai, A., Mele, M., 2013. Dietary olive cake reduces the oxidation of lipids, including cholesterol, in lamb meat enriched in polyunsaturated fatty acids. Meat Sci. 93, 703–714.

Lutterodt, H., Slavin, M., Whent, M., Turner, E., Yu, L., 2011. Fatty acid composition, oxidative stability, antioxidant and antiproliferative properties of selected cold-pressed grape seed oils and flours. Food Chem. 128, 391–399.

Makkar, H.P.S., 2003. Effects and fate of tannins in ruminant animals, adaptation to tannins, and strategies to overcome detrimental effects of feeding tannin-rich feeds. Small Rumin. Res. 49, 241–256.

Makkar, H.P.S., Singh, B., 1991. Effect of drying conditions on tannin, fiber and lignin levels in mature oak (*Quercus-Incana*) leaves. J. Sci. Food Agric. 54, 323–328.

Manso, T., Gallardo, B., Guerra-Rivas, C., 2016. Modifying milk and meat fat quality through feed changes. Small Rumin. Res. 142, 31–37.

Mao, Y., Hopkins, D.L., Zhang, Y., Luo, X., 2016. Consumption patterns and consumer attitudes to beef and sheep meat in China. Am. J. Food Nutr. 4, 30–39.

Mapiye, C., Aldai, N., Turner, T.D., Aalhus, J.L., Rolland, D.C., Kramer, J.K.G., Dugan, M.E.R., 2012. The labile lipid fraction of meat: From perceived disease and waste to health and opportunity. Meat Sci. 92, 210–220.

Martinez-Fernandez, G., Denman, S.E., Cheung, J., McSweeney, C.S., 2017. Phloroglucinol degradation in the rumen promotes the capture of excess hydrogen generated from methanogenesis inhibition. Front. Microbiol. 8, 1871.

McAfee, A.J., McSorley, E.M., Cuskelly, G.J., Moss, B.W., Wallace, J.M.W., Bonham, M.P., Fearon, A.M., 2010. Red meat consumption: An overview of the risks and benefits. Meat Sci. 84, 1–13.

McDonald, P., Edwards, R.A., Greenhalgh, J.F.D., Morgan, C.A., Sinclair, L.A., Wilkinson, R.G., 2011. Animal Nutrition, 7th ed. Pearson Education Limited, Harlow, United Kingdom.

McGrath, J., Duval, S.M., Tamassia, L.F.M., Kindermann, M., Stemmler, R.T., de Gouvea, V.N., Acedo, T.S., Immig, I., Williams, S.N., Celi, P., 2018. Nutritional strategies in ruminants: A lifetime approach. Res. Vet. Sci. 116, 28–39.

Meissner, H.H., 1997. Recent research on forage utilization by ruminant livestock in South Africa.

Anim. Feed Sci. Technol. 69, 103–119.

Meissner, H.H., Scholtz, M.M., Engelbrecht, F.A., 2013. Sustainability of the South African livestock sector towards 2050 part 2: Challenges, changes and required implementations. S. Afr. J. Anim. Sci. 43, 298–319.

Mertens, D.R., 1994. Regulation of forage Intake, in: Forage Quality, Evaluation, and Utilization. American Society of Agronomy, Crop Science Society of America, Soil Science Society of America, Madison, WI, pp. 494–532.

Moate, P.J., Williams, S.R.O., Torok, V.A., Hannah, M.C., Ribaux, B.E., Tavendale, M.H., Eckard, R.J., Jacobs, J.L., Auldist, M.J., Wales, W.J., 2014. Grape marc reduces methane emissions when fed to dairy cows. J. Dairy Sci. 97, 5073–5087.

Molina-Alcaide, E., Moumen, A., Martín-García, A.I., 2008. Byproducts from viticulture and the wine industry: Potential as sources of nutrients for ruminants. J. Sci. Food Agric. 88, 597–604.

Montossi, F., Font-i-Furnols, M., del Campo, M., San Julián, R., Brito, G., Sañudo, C., 2013. Sustainable sheep production and consumer preference trends: Compatibilities, contradictions, and unresolved dilemmas. Meat Sci. 95, 772–789.

Moore, K.J., Jung, H.-J.G., 2001. Lignin and fiber digestion. J. Range Manag. 54, 420–430.

Mueller-Harvey, I., Mlambo, V., Sikosana, J.L.N., Smith, T., Owen, E., Brown, R.H., 2007. Octanol−water partition coefficients for predicting the effects of tannins in ruminant nutrition. J. Agric. Food Chem. 55, 5436–5444.

Naziri, E., Nenadis, N., Mantzouridou, F.T., Tsimidou, M.Z., 2014. Valorization of the major agrifood industrial byproducts and waste from Central Macedonia (Greece) for the recovery of compounds for food applications. Food Res. Int. 65, 350–358.

Newell, D.G., Koopmans, M., Verhoef, L., Duizer, E., Aidara-Kane, A., Sprong, H., Opsteegh, M.,

Langelaar, M., Threfall, J., Scheutz, F., der Giessen, J. van, Kruse, H., 2010. Food-borne diseases - The challenges of 20 years ago still persist while new ones continue to emerge. Int. J. Food Microbiol. 139, S3–S15.

National Research Council, 2007. Nutrient Requirements of Small Ruminants. National Academy Press, Washington DC, USA.

OECD (Organization for Economic Co-operation and Development), 2016. Meat consumption (indicator). https://data.oecd.org/agroutput/meat-consumption.htm (accessed 9.13.16).

Ortuño, J., Serrano, R., Bañón, S., 2015. Antioxidant and antimicrobial effects of dietary supplementation with rosemary diterpenes (carnosic acid and carnosol) vs vitamin E on lamb meat packed under protective atmosphere. Meat Sci. 110, 62–69.

Papuc, C., Goran, G. V., Predescu, C.N., Nicorescu, V., Stefan, G., 2017. Plant polyphenols as antioxidant and antibacterial agents for shelf-life extension of meat and meat products: Classification, structures, sources, and action mechanisms. Compr. Rev. Food Sci. Food Saf. 16, 1243–1268.

Patra, A.K., Saxena, J., 2010. A new perspective on the use of plant secondary metabolites to inhibit methanogenesis in the rumen. Phytochemistry 71, 1198–1222.

Patra, A.K., Saxena, J., 2009. Dietary phytochemicals as rumen modifiers: A review of the effects on microbial populations. Antonie Van Leeuwenhoek 96, 363–375.

Pisoschi, A.M., Pop, A., Georgescu, C., Turcuş, V., Olah, N.K., Mathe, E., 2018. An overview of natural antimicrobials role in food. Eur. J. Med. Chem. 143, 922–935.

Planinić, M., Aliakbarian, B., Perego, P., Greganić, K., Tomas, S., Bucić-Kojić, A., 2015. Influence of Temperature and Drying Time on Extraction Yield of Phenolic Compounds from Grape Pomace Variety “Portogizac.” Chem. Biochem. Eng. Q. 29, 343–350.

Priolo, A., Micol, D., Agabriel, J., Prache, S., Dransfield, E., 2002. Effect of grass or concentrate

feeding systems on lamb carcass and meat quality. Meat Sci. 62, 179–185.

Priolo, A., Vasta, V., 2007. Effects of tannin-containing diets on small ruminant meat quality. Ital. J. Anim. Sci. 6, 527–530.

Rani, Z.T., Hugo, A., Hugo, C.J., Vimiso, P., Muchenje, V., 2017. Effect of post-slaughter handling during distribution on microbiological quality and safety of meat in the formal and informal sectors of South Africa: A review. S. Afr. J. Anim. Sci. 47, 255–267.

Ribeiro, L.F., Ribani, R.H., Francisco, T.M.G., Soares, A.A., Pontarolo, R., Haminiuk, C.W.I., 2015. Profile of bioactive compounds from grape pomace (*Vitis vinifera* and *Vitis labrusca*) by spectrophotometric, chromatographic and spectral analyses. J. Chromatogr. B 1007, 72–80.

Rodríguez-Serrano, T.M., Panea, B., Alcalde, M.J., 2016. A European vision for the small ruminant sector. Promotion of meat consumption campaigns. Small Rumin. Res. 142, 3–5.

Sañudo, C., Muela, E., Del Mar Campo, M., 2013. Key factors involved in lamb quality from farm to fork in Europe. J. Integr. Agric. 12, 1919–1930.

Scalbert, A., 1991. Antimicrobial properties of tannins. Phytochemistry 30, 3875–3883.

Shi, J., Yu, J., Pohorly, J.E., Kakuda, Y., 2003. Polyphenolics in grape seeds- biochemistry and functionality. J. Med. Food 6, 291–299.

Sui, Y., Yang, J., Ye, Q., Li, H., Wang, H., 2014. Infrared, convective, and sequential Infrared and convective drying of wine grape pomace. Dry. Technol. 32, 686–694.

Sun, X.D., Holley, R.A., 2012. Antimicrobial and antioxidative strategies to reduce pathogens and extend the shelf life of fresh red meats. Compr. Rev. Food Sci. Food Saf. 11, 340–354.

Tayengwa, T., Mapiye, C., 2018. Citrus and winery wastes: promising dietary supplements for sustainable ruminant animal nutrition, health, production, and meat quality. Sustainability 10, 3718.

Thornton, P.K., 2010. Livestock production: Recent trends, future prospects. Philos. Trans. R. Soc.

B Biol. Sci. 365, 2853–2867.

Tseng, A., Zhao, Y., 2012. Effect of different drying methods and storage time on the retention of bioactive compounds and antibacterial activity of wine grape pomace (Pinot Noir and Merlot). J. Food Sci. 77, H192–H201.

Waghorn, G., 2008. Beneficial and detrimental effects of dietary condensed tannins for sustainable sheep and goat production-Progress and challenges. Anim. Feed Sci. Technol. 147, 116–139.

Winkler, A., Weber, F., Ringseis, R., Eder, K., Dusel, G., 2015. Determination of polyphenol and crude nutrient content and nutrient digestibility of dried and ensiled white and red grape pomace cultivars. Arch. Anim. Nutr. 69, 187–200.

Yu, J., Ahmedna, M., 2013. Functional components of grape pomace: Their composition, biological properties and potential applications. Int. J. Food Sci. Technol. 48, 221–237.

Zalikarenab, L., Pirmohammadi, R., Teimuriyansari, A., 2007. Chemical composition and digestibility of dried white and red grape pomace for ruminants. J. Anim. Vet. Adv. 6, 1107–1111.

Zepf, F., Jin, B., 2013. Bioconversion of grape marc into protein rich animal feed by microbial fungi. Chem. Eng. Process Tech. 1, 1011.

Zhang, L., Wang, Y., Li, D., Ho, C.-T., Li, J.-S., Wan, X., 2016. The absorption, distribution, metabolism and excretion of procyanidins. Food Funct. 7, 1273–1281.

Zhang, N., Hoadley, A., Patel, J., Lim, S., Li, C., 2017. Sustainable options for the utilization of solid residues from wine production. Waste Manag. 60, 173–183.

Zhao, J.X., Li, Q., Zhang, R.X., Liu, W.Z., Ren, Y.S., Zhang, C.X., Zhang, J.X., 2018. Effect of dietary grape pomace on growth performance, meat quality and antioxidant activity in ram lambs. Anim. Feed Sci. Technol. 236, 76–85.

Chapter 3 Impact of preservation technique on nutrient composition and in vitro ruminal digestibility of pomace from three grape varieties[2]

ABSTRACT

Grape pomace, a byproduct of the winemaking industry is a potential source of animal feed, but its nutrient and chemical composition is not sufficiently studied in South Africa. The current study investigated the changes in nutrient composition and *in vitro* ruminal digestibility of neutral detergent fiber of grape (*Vitis vinifera* L. cv. Pinotage, Shiraz and Sauvignon Blanc) pomace after three drying treatments: sun-drying (7d), oven-drying (72 h at 60 °C) and freeze-drying (72 h). Oven-dried Shiraz had the highest dry matter (DM), crude protein (CP) and the least ash content ($P \leq 0.05$), while sun-dried Shiraz had the highest neutral detergent fiber (aNDFom), acid detergent fiber (ADFom) and lignin compared to other drying × variety interactions ($P \leq 0.05$). Freeze-dried Sauvignon Blanc had the highest starch content, whereas freeze-dried Shiraz had the highest ether extract (EE) content

[2] A version of this chapter has been published:

Chikwanha, O.C., Raffrenato, E., Muchenje, V., Musarurwa, H.T., Mapiye, C., 2018. Varietal differences in nutrient, amino acid and mineral composition and in vitro rumen digestibility of grape (Vitis vinifera) pomace from the Cape Winelands vineyards in South Africa and impact of preservation techniques. Ind. Crops Prod. 118, 30–37. doi:10.1016/j.indcrop.2018.03.026

relative to other drying × variety interactions ($P \leq 0.05$). The *in vitro* ruminal digestibility of aNDFom at 24 and 48 h were highest in freeze-dried Pinotage followed by freeze-dried Shiraz and Sauvignon Blanc drying × variety interactions ($P \leq 0.05$). Freeze-dried Pinotage had the majority of amino acids in highest concentration, followed by freeze-dried Shiraz ($P \leq 0.05$). Freeze- and oven-dried Pinotage exhibited higher contents of potassium, magnesium, sulfur, sodium, iron and aluminum relative to other drying × variety interactions. Overall, sun- and oven-dried Shiraz had the highest DM, CP, aNDFom, ADFom and lignin contents, freeze- and oven-dried Pinotage had the highest mineral composition, while freeze-dried Pinotage had the best amino acid profile and *in vitro* ruminal digestibility of aNDFom at 24 and 48 h.

Key words

Drying method, Grape pomace, *In vitro* digestibility, Nutrient composition.

3.1 Introduction

The wine growing region of the Western Cape Province of South Africa has a Mediterranean climate, ideal for the wine grape, *Vitis vinifera* L. The South African wine industry produces close to 1.5 million tons of grapes per annum (SAWIS, 2017) of which 250 g/ kg DM ends up as grape pomace (GP) after juice or wine extraction (Yu and Ahmedna, 2013). The quantity of pomace, however, varies with grape cultivar, pressing process and fermentation step (Beres et al., 2017). The main constituents of GP are skins, seeds and stems produced after pressing the crushed grapes in white wine production or fermented grapes in red wine production (Maier et al., 2009; Mendes et al., 2013; Naziri et al., 2014; Spigno et al., 2013).

Traditionally, use of GP for economic and environmental reasons has focused on recycling for soil conditioning, recovery of bioactive compounds and production of ethanol, flour, food colorings, grape seed oil and animal feed (Arvanitoyannis et al., 2006; Beres et al., 2017; Brenes et al., 2016; García-Lomillo and González-SanJosé, 2017). These applications have limited markets and can absorb only a small portion of the generated GP waste, therefore a large proportion of the GP that is produced is discarded (El Achkar et al., 2016). Disposal of GP is usually carried out by external companies, thus adding to the production costs for the wine industry (Spigno et al., 2017). Besides economic costs, there are many environmental costs associated with the disposal of GP. For example, the application of GP as an organic fertilizer can have phenolic content related phytotoxic effects that inhibit seed germination and root growth (Barbera et al., 2013; Devesa-Rey et al., 2011). The low pH of GP has been linked to delayed mineralization and long-term loss of carbonate from the top soil (Barbera et al., 2013; Nendel and Reuter, 2007), which can have negative effects on soil chemistry. Other environmental problems include surface and groundwater pollution, foul odors, attraction of flies and pests that may spread diseases, and leachates of tannins and other compounds that can result in oxygen depletion in the soil and ground waters, affecting surrounding flora and fauna (Arvanitoyannis et al., 2006; Dwyer et al., 2014). Thus, identification of alternative use for GP can possibly result in decrease in the amount that is disposed and consequently reduce environmental problems.

Grape pomace has been included in ruminant diets either in the fresh, dried or ensiled form mostly to meet maintenance requirements (Baumgärtel et al., 2007). Wet GP typically has high moisture content (500 – 720 g/ kg DM) and water activity, which makes it highly perishable (Goula et al., 2016). The seasonality of GP availability, high energy costs for dehydration, variable chemical composition and presence of undesirable contaminants, such as mycotoxins, biogenic amines, pesticides and heavy metals (e.g., cadmium and lead) (Bustamante et al., 2008; Moncalvo et al., 2016)

often pose challenges for its utilization as an animal feed. Nutritional composition and digestibility of GP is mainly influenced by environmental conditions, grape variety (Baumgärtel et al., 2007; Spanghero et al., 2009; Rondeau et al., 2013) and drying methods (Basalan et al., 2011; Zalikarenab et al., 2007) among other factors. The effects of different drying methods on nutrient and chemical composition of GP from locally grown varieties in South Africa, particularly the locally bred variety, Pinotage, are not known. Lack of such fundamental information currently limits the inclusion of GP in ruminant diets. The objective of the current study was, therefore, to evaluate the effects of drying method on nutrient and chemical composition and *in vitro* neutral detergent fiber digestibility (ivNDFd) of pomace from three grape varieties commonly grown in South Africa.

3.2 Materials and methods

3.2.1 Preparation of grape pomace samples

The three most commonly produced grape (*Vitis vinifera* L.) varieties (i.e., Pinotage, Sauvignon Blanc and Shiraz) in South Africa were sourced at Stellenbosch University's Welgevallen Experimental farm (Stellenbosch, South Africa). Sauvignon Blanc is a white grape variety, while Pinotage and Shiraz are red varieties. Pinotage was harvested in January, Sauvignon Blanc in February and Shiraz in March 2017. All the varieties were harvested over six consecutive days (6 pressings). Each day, about eight tons of each variety were harvested, pressed and a representative sample (2 kg) of fresh pomace collected (n = 6 pressings). The sample from each day's pressing for each variety was divided into three fractions of 500 g and randomly allocated to three drying treatments: sun-drying for 7 days at temperatures between 25 and 33 °C, oven drying at 60 °C for 72 h and freeze-drying for 72 h (vacuum pressure of 7 mTorr and condenser temperature of -88.7 °C; VirTis Co., Gardiner, NY, USA). The dried samples were ground into fine powders using a Wiley

mill (Model 4, Thomas Scientific, Swedesboro, NJ, USA) with a 1 mm sieve and stored at -20 °C pending analyses.

3.2.2 Chemical analyses

Dry matter (method 934.01), ash (method 942.05) and ether extract (EE; method 920.39) contents were determined according to the AOAC (2002) procedures. Total nitrogen content was analyzed using the Dumas method with a macro-Nitrogen analyzer (LECO® FP528, LECO Corporation, Miami, USA). Crude protein (CP) was calculated by multiplying the nitrogen content by a factor of 6.25. Starch was measured using a commercial assay (Total Starch Megazyme kit KTSTA, Megazyme International Ireland Ltd., Wicklow, Ireland), following the method for samples containing glucose and/ or maltodextrins (Hall, 2009). Neutral detergent fiber (aNDFom) was determined using heat-stable alpha-amylase and addition of sodium sulfite (Mertens et al., 2002). Acid detergent fiber (ADFom) was performed according to AOAC (2002). Lignin (sa.) was analyzed according to Goering and Van Soest (1970) as modified by Raffrenato and Van Amburgh (2011). Neutral detergent fiber, ADFom and lignin (sa.) were expressed exclusive of ash. All chemical analyses were performed in triplicate.

3.2.3 In vitro ruminal digestibility

Two rumen-cannulated Holstein dairy cows were used as rumen content donors for the *in vitro* ruminal NDF (ivNDF) digestibility. The animals were fed a total mixed ration consisting of lucerne hay (40%) and concentrates (60%) at 07:00 and 16:00. Rumen fluid from each cow was collected before the morning feeding and mixed in pre-warmed insulated Thermos flask. The rumen fluid was filtered through four layers of cheesecloth, 100 µm mesh and glass wool prior to inoculation. Ground and dried GP (~0.5 g) was weighed into 125 mL Erlenmeyer flask (in duplicate) before the addition

of 40 mL of Van Soest buffer as described by Goering and Van Soest (1970). The flasks were placed in a heated (39.5 °C) shaking water bath under CO_2 positive pressure to ensure an anaerobic environment, before addition of 10 mL of rumen fluid. *In vitro* ruminal NDF digestibility was estimated as the difference between the NDF weight incubated and the NDF weight of the filter obtained using 50 mL sintered Gooch crucible porosity 2, with added Whatman glass microfiber filters (934-AH®, GE Healthcare, Pittsburgh, PA, USA), expressed as a proportion of DM weight incubated. Residual NDF were measured at 24 and 48 h. The experimental unit was each individual incubation. The analyses were performed in two runs, with each treatment analyzed in duplicate.

3.2.4 Amino acid analyses

Amino acid separation and detection was performed using Waters Acquity Ultra Performance Liquid Chromatograph fitted with a photodiode array detector. Briefly, 100 mg of dried ground GP was used for the extraction of amino acids using acid hydrolysis extraction (0.5 mL of 6M HCl). L-Norvaline was used as the standard amino acid. Derivatization of the amino acids was performed using AccQ Fluor reagent Kit, Waters (En Yvelines Cedex, France). For derivatization, 10 µL of standard/ sample was mixed in vials with 70 µL buffer solution (0.2M borate buffer) and 20 µL of derivatization reagent (2 mg/ mL AQC). The capped vials were transferred into an oven at 55 °C for 10 minutes to build stable derivatives. A volume of 1 µL of standard/ sample solution was injected into the mobile phase onto the Waters UltraTag C18 column (2.1 m × 50 mm × 1.7 µm) at 60 °C. Analytes eluting off the column were detected by the PDA detector and each amino acid determined based on the retention time in the column. Data acquisition was performed by MassLynx V4.1 2011 software (Waters, Milford, USA). The peak areas and retention times were used to plot calibration curves and subsequent quantification of amino acid concentration. All analyses were performed in triplicate.

3.2.5 Mineral analyses

The dried GP was first exposed to microwave acid digestion and dissolution of the sample. Minerals were quantified by inductively coupled plasma-atomic emission spectrometry according to Sah and Miller (1992). All analyses were performed in triplicate.

3.2.6 Statistical analyses

The data was analyzed as a completely randomized designed with a 3 × 3 factorial arrangement of treatments using the generalized linear model procedure of SAS (2012). The total number of observations for nutrient composition data were 162 (3 drying methods × 3 varieties × 6 pressings × 3 replications). The total number of observations for *in vitro* ruminal NDF digestibility were 216 (3 drying methods × 3 varieties × 6 pressings × 2 runs × 2 run replications). After averaging replications for nutrient composition, and the runs and run replications for *in vitro* ruminal NDF digestibility, the remaining 54 observations were subjected to analysis of variance using the following model:

$$Y_{ijk} = \mu + D_i + V_j + (DV)_{ij} + \varepsilon_{ijk}$$

Where:

Y_{ijk} = response variable (nutrient composition, *in vitro* ruminal NDF digestibility),

μ = overall mean,

D_i = effect of the i^{th} drying method (i = sun, oven, freeze),

V_j = effect of j^{th} variety (j = Pinotage, Sauvignon Blanc, Shiraz),

$(DV)_{ij}$ = interaction of i^{th} drying method and j^{th} variety and,

ε_{ijk} = residual error.

Treatment means were generated and separated using the LSMEANS and PDIFF options, respectively. The significance threshold for all statistical analyses was set at $P \leq 0.05$ and a tendency when $0.05 < P \leq 0.10$.

3.3 Results

3.3.1 Nutrient content of grape pomace

Table 3.1 shows the nutrient content for the treatments and their interactions. The interaction effects were significant for all the analyzed proximate components parameters ($P \leq 0.05$). Oven-dried Pinotage and Shiraz had the highest DM ($P \leq 0.05$) followed by sun-dried Pinotage and Shiraz. Sun-dried Pinotage had the greatest ash content, followed by oven-dried Pinotage ($P \leq 0.05$). Compared to other GP drying × variety interactions, oven-dried Shiraz had the highest CP values ($P \leq 0.05$), followed by freeze- and sun-dried Shiraz with no significant difference between them ($P > 0.05$). Sauvignon Blanc, irrespective of the drying methods had the lowest CP values while Pinotage had intermediate values ($P \leq 0.05$). Freeze-dried Sauvignon Blanc, relative to other drying × variety interactions had the highest starch content ($P \leq 0.05$) followed by sun- and oven-dried Sauvignon Blanc with no significant difference between them ($P > 0.05$). The EE content was highest for freeze-dried Shiraz followed by sun- and oven-dried Shiraz, respectively ($P \leq 0.05$). Overall, Pinotage had intermediate EE values whereas Sauvignon Blanc had lower values regardless of drying method ($P \leq 0.05$).

Sun-dried Shiraz had the highest aNDFom and ADFom followed by oven- and freeze-dried Shiraz ($P \leq 0.05$). Irrespective of drying method, Pinotage had intermediate values for aNDFom and ADFom, whereas Sauvignon Blanc had lower values with a similar drying method trend reported for Shiraz ($P \leq 0.05$). Lignin (sa.) was highest for sun-dried Shiraz ($P \leq 0.05$), followed by sun-dried Pinotage and oven-dried Shiraz with no significant difference between them ($P > 0.05$). Shiraz had intermediate values for lignin (sa.), while Sauvignon Blanc had the lowest regardless of drying method ($P \leq 0.05$).

3.3.2 In vitro NDF digestibility of grape pomace

The results for *in vitro* ruminal NDF digestibility are presented in Table 3.1. Variety had no effect ($P > 0.05$) on ivNDF digestibility but drying method and its interaction influenced ($P \leq 0.05$) ivNDF digestibility. Freeze-dried Pinotage had the highest ivNDF digestibility at 24 and 48 h incubations ($P \leq 0.05$) followed by freeze-dried Shiraz and Sauvignon Blanc with no significant difference between these two drying × variety interactions ($P > 0.05$). Oven-dried Shiraz had intermediate 24 h ivNDF digestibility values followed by sun-dried Shiraz and Sauvignon Blanc ($P \leq 0.05$) with no significant difference between them ($P > 0.05$). A similar trend was observed for the 48 h ivNDF digestibility with oven-dried Pinotage and Shiraz having intermediate values with no significant difference between them ($P > 0.05$) followed by sun-dried Sauvignon Blanc ($P \leq 0.05$).

Table 3.1 Effects of drying method, grape pomace variety and their interactions on nutritional composition and *in vitro* ruminal digestibility (g/ kg DM)

Item	Sun drying			Oven drying			Freeze drying			SEM	P value		
	Pinotage	Shiraz	S. Blanc*	Pinotage	Shiraz	S. Blanc	Pinotage	Shiraz	S. Blanc		D	V	D × V
DM	926^{b}	924^{b}	904^{e}	930^{a}	929^{a}	916^{c}	912^{d}	914^{cd}	893^{f}	0.10	<0.01	<0.01	<0.01
Ash	66.5^{a}	53.2^{d}	60.0^{c}	63.6^{b}	51.0^{e}	58.5^{c}	59.6^{c}	53.0^{d}	59.4^{c}	0.68	<0.01	<0.01	<0.01
Crude protein	124^{c}	130^{b}	89.0^{f}	114^{d}	134^{a}	90.7^{f}	109^{e}	131^{b}	90.9^{f}	0.49	<0.01	<0.01	<0.01
Starch	55.5^{f}	63.5 ^{d}	216^{b}	59.1^{e}	65.6^{d}	216^{b}	62.6 ^{d}	72.6^{c}	235^{a}	1.36	0.32	<0.01	<0.01
Ether extract	68.8^{e}	95.2^{b}	52.8^{f}	67.9^{e}	90.3^{c}	49.9^{g}	77.2^{d}	106^{a}	49.4^{g}	0.65	<0.01	<0.01	<0.01
aNDFom	322^{d}	389^{a}	256^{g}	312^{e}	366^{b}	243^{h}	303^{f}	339^{c}	2 19^{i}	2.51	<0.01	<0.01	<0.01
ADFom	314^{d}	414^{a}	251^{g}	306^{e}	336^{b}	242^{h}	277^{f}	328^{c}	213^{i}	2. 90	<0.01	<0.01	<0.01
ADL	198^{b}	222^{a}	171^{f}	192^{c}	201^{b}	177^{e}	185^{d}	186^{d}	169^{f}	2.82	<0.01	<0.01	<0.01
24 h ivNDFd	244^{e}	258^{d}	258^{d}	246^{e}	264^{c}	235 ^{f}	285^{a}	276^{b}	276^{b}	3.92	0.26	0.05	<0.01
48 h ivNDFd	269^{e}	273^{e}	294^{d}	310^{c}	313^{c}	295^{d}	338^{a}	316^{b}	318^{b}	2.55	0.04	<0.01	0.09

Least square means with different superscript within a row are significantly different ($P \leq 0.05$).
*S. Blanc - Sauvignon Blanc.
D, drying method.
V, variety.
D × V, drying × variety interaction.

3.3.3 Amino acid profile of grape pomace

Table 3.2 shows the amino acid profiles of the drying × grape variety interactions. The content of all the tested amino acids had significant interactions ($P \leq 0.05$). Generally, freeze-dried Pinotage had the highest amino acid composition for alanine, arginine, aspartic acid, cysteine, glutamic acid, glycine, isoleucine, leucine, lysine, serine and valine followed by freeze-dried Shiraz and Sauvignon Blanc ($P \leq 0.05$). High levels of asparagine, phenylalanine, proline and tyrosine were observed for freeze-dried Shiraz compared to other drying × variety interactions ($P \leq 0.05$). Glutamine was relatively high for freeze-dried Sauvignon Blanc, followed by sun- and oven-dried Sauvignon Blanc, respectively ($P \leq 0.05$). In comparison to other drying × variety interactions, histidine was highest for freeze-dried Pinotage and freeze-dried Shiraz ($P \leq 0.05$) with no significant difference between them, followed by freeze-dried Sauvignon Blanc. Methionine was highest for sun-dried Sauvignon Blanc ($P \leq 0.05$) followed by freeze-dried Sauvignon Blanc and oven-dried Sauvignon Blanc, with no significant difference between them ($P > 0.05$). Sun-dried Pinotage and Shiraz had the highest threonine content compared to other drying × variety interactions ($P \leq 0.05$) but there was no significant difference between the two drying × variety interactions.

3.3.4 Mineral content of grape pomace

The mineral content for the treatments and their interactions are presented in Table 3.3. Interactions were significant for all the detected minerals ($P \leq 0.05$). Calcium content was highest for freeze- and sun- dried Shiraz, with no significant difference between them followed by oven- and freeze-dried Pinotage, respectively ($P \leq 0.05$). Phosphorus content was highest for freeze-dried Shiraz ($P \leq 0.05$) followed by sun-dried and oven-dried Shiraz, respectively ($P > 0.05$). Regardless of drying method, Pinotage generally had higher potassium content compared to other

varieties ($P \leq 0.05$). Overall, the highest content of magnesium, sulfur, sodium, iron and aluminum were observed for freeze- and oven-dried Pinotage compared to other drying × variety interactions ($P \leq 0.05$). Relative to other drying × variety interactions, manganese was highest for sun-dried Sauvignon Blanc, followed by freeze-dried Sauvignon Blanc and sun-dried Shiraz ($P \leq 0.05$) with no significant differences between them ($P \leq 0.05$). Copper was highest for the Shiraz variety regardless of drying method ($P \leq 0.05$).

Table 3.2 Effects of drying method, grape pomace variety and their interactions on amino acid composition (mg/ g DM)

Item	Sun drying			Oven drying			Freeze drying			SEM	P value		
	Pinotage	Shiraz	S. Blanc*	Pinotage	Shiraz	S. Blanc*	Pinotage	Shiraz	S. Blanc*		D	V	D × V
Alanine	4.25^{d}	4.86^{c}	4.83^{c}	4.11^{e}	3.75^{g}	3.90^{f}	5.97^{a}	5.12^{b}	5.11^{b}	0.016	0.01	<0.01	<0.01
Arginine	7.10^{h}	7.81^{e}	8.99^{b}	7.27^{f}	8.03^{d}	7.23^{g}	11.0^{a}	8.99^{b}	8.60^{c}	0.017	<0.01	<0.01	<0.01
Asparagine	1.76^{g}	2.12^{d}	2.32^{c}	1.87^{f}	1.48^{h}	1.92^{e}	2.31^{c}	2.57^{a}	2.41^{b}	0.009	<0.01	<0.01	<0.01
Aspartic acid	7.49^{g}	8.26^{e}	8.38^{d}	7.74^{f}	6.39^{i}	6.52^{h}	10.4^{a}	9.16^{b}	9.03^{c}	0.012	<0.01	<0.01	<0.01
Cysteine	0.11^{f}	0.18^{c}	0.14^{d}	0.13^{e}	0.13^{e}	0.07^{g}	0.45^{a}	0.28^{b}	0.28^{b}	0.003	<0.01	<0.01	<0.01
Glutamic acid	12.8^{g}	14.0^{e}	14.5^{d}	13.1^{f}	10.2^{i}	10.5^{h}	20.6^{a}	17.60^{b}	17.10^{c}	0.015	<0.01	<0.01	<0.01
Glutamine[1]	20.8^{e}	24.23^{d}	40.2^{b}	17.69^{f}	19.84^{f}	38.8^{c}	22.4^{d}	21.2^{e}	49.6^{a}	1.090	<0.01	<0.01	<0.01
Glycine	8.02^{e}	9.15^{d}	9.12^{d}	5.89^{h}	6.18^{g}	7.41^{f}	12.1^{a}	11.7^{b}	10.6^{c}	0.015	0.06	<0.01	<0.01
Histidine	3.18^{e}	3.78^{d}	3.87^{c}	2.74^{g}	3.11^{f}	3.29^{d}	5.04^{a}	5.04^{a}	4.74^{b}	0.017	<0.01	<0.01	<0.01
Isoleucine	3.59^{f}	3.93^{e}	4.01^{d}	3.05^{h}	3.03^{h}	3.43^{g}	4.94^{a}	4.65^{b}	4.56^{c}	0.015	0.01	<0.01	<0.01
Leucine	6.27^{f}	7.13^{e}	7.25^{d}	5.38^{h}	5.37^{h}	6.05^{g}	8.97^{a}	8.33^{b}	8.05^{c}	0.015	0.31	<0.01	<0.01
Lysine	3.28^{f}	3.89^{c}	3.96^{c}	3.39^{e}	3.78^{d}	2.67^{g}	6.56^{a}	5.02^{b}	4.99^{b}	0.024	<0.01	<0.01	<0.01
Methionine	0.35^{g}	0.53^{d}	0.83^{a}	0.49^{e}	0.52^{d}	0.76^{b}	0.39^{f}	0.57^{c}	0.74^{b}	0.010	<0.01	<0.01	<0.01
Phenylalanine	5.88^{f}	6.69^{c}	6.26^{d}	4.43^{h}	4.53^{g}	5.93^{e}	5.99^{d}	7.41^{a}	6.86^{b}	0.015	<0.01	<0.01	<0.01
Proline	7.88^{e}	9.36^{c}	8.66^{d}	4.70^{i}	5.24^{h}	5.61^{g}	7.29^{f}	10.5^{a}	10.1^{b}	0.038	<0.01	<0.01	<0.01
Serine	4.55^{f}	5.05^{e}	5.36^{d}	4.21^{g}	3.98^{h}	4.23^{g}	7.60^{a}	5.91^{c}	5.99^{b}	0.016	<0.01	<0.01	<0.01
Threonine	41.1^{a}	41.1^{a}	33.5^{c}	20.1^{f}	27.6^{d}	38.8^{b}	24.6^{e}	33.4^{c}	32.6^{c}	0.019	0.01	<0.01	<0.01
Tyrosine	4.43^{f}	5.23^{c}	5.43^{b}	3.56^{g}	3.48^{h}	4.86^{d}	4.75^{e}	5.84^{a}	5.40^{b}	0.024	<0.01	<0.01	<0.01
Valine	4.24^{f}	4.67^{d}	4.57^{e}	3.80^{h}	3.59^{i}	4.07^{g}	5.87^{a}	5.59^{b}	5.45^{c}	0.016	0.01	<0.01	<0.01

Least square means with different superscript within a row are significantly different ($P \leq 0.05$).
[1] Glutamine content was expressed as µg/ g DM.
*S. Blanc - Sauvignon Blanc.
D, drying method.
V, variety.
D × V, drying × variety interaction.

Table 3.3 Effects of drying method, grape pomace variety and their interactions on mineral composition (g/ kg DM)

Item	Sun drying			Oven drying			Freeze drying			SEM	P value		
	Pinotage	Shiraz	S. Blanc*	Pinotage	Shiraz	S. Blanc	Pinotage	Shiraz	S. Blanc		D	V	D × V
Calcium	2.63^{f}	3.72^{a}	2.29^{h}	3.62^{b}	3.24^{d}	2.39^{g}	3.36^{c}	3.73^{a}	2.68^{e}	0.05	<0.01	<0.01	<0.01
Phosphorus	2.33^{f}	3.25^{b}	2.32^{f}	2.49^{d}	2.83^{c}	2.20^{g}	2.42^{e}	3.42^{a}	2.17^{h}	0.05	<0.01	<0.01	<0.01
Potassium	24.1^{b}	22.3^{c}	15.0^{g}	24.2^{b}	21.5^{d}	17.6^{f}	25.3^{a}	23. 6^{b}	18.3^{e}	0.41	<0.01	<0.01	<0.01
Magnesium	1.12^{d}	1.21^{c}	0.97^{f}	1.35^{a}	1.05^{e}	0.95^{f}	1.37^{a}	1.29^{b}	1.05^{e}	0.03	<0.01	<0.01	<0.01
Sulfur	1.35^{c}	1.15^{d}	1.02^{e}	1.54^{a}	1.37^{bc}	0.97^{f}	1.54^{a}	1.39^{b}	1.02^{e}	0.02	<0.01	<0.01	<0.01
Sodium[1]	98.6^{b}	49.3^{c}	32.8^{e}	121^{a}	35.7^{e}	33.2^{e}	117^{a}	40.7^{d}	34.8^{e}	2.42	0.02	<0.01	<0.01
Zinc[1]	11.5^{f}	14.6^{d}	7.3^{h}	15.1^{c}	13.5^{e}	9.0^{g}	16.6^{b}	19.2^{a}	6.9^{h}	0.34	<0.01	<0.01	<0.01
Manganese[1]	14.2^{g}	17.7^{b}	18.5^{a}	15.9^{de}	15.4^{f}	15.6^{ef}	16.1^{d}	17.1^{c}	17.6^{b}	0.41	0.001	<0.01	<0.01
Iron[1]	147^{b}	115^{c}	115^{c}	166^{a}	78.9^{f}	88.8^{e}	170^{a}	94.8^{d}	84.1^{e}	2.92	<0.01	<0.01	<0.01
Copper[1]	7.93^{cd}	13.4^{a}	7.99^{c}	9.08^{b}	13.1^{a}	6.75^{cd}	8.88^{b}	13.1^{a}	7.63^{d}	0.31	0.61	<0.01	<0.02
Aluminum[1]	152^{b}	51.1^{d}	67.8^{c}	219^{a}	53.2^{d}	61.5^{c}	221^{a}	48.7^{d}	60.8^{c}	3.60	0.01	<0.01	<0.01

Least square means with different superscript within a row are significantly different ($P \leq 0.05$).
*S. Blanc - Sauvignon Blanc.
[1], mg/ kg DM
D, drying method.
V, variety.
D × V, drying × variety interaction.

3.4 Discussion

The relatively high DM content observed for oven-dried Pinotage and Shiraz could be related to varietal and drying method differences. The varietal differences could be because of presence of volatile compounds dominant in the red varieties, which volatize during prolonged drying. De Torres et al. (2010) reported a marked reduction of volatiles (i.e., terpenes, C6 alcohols and aldehydes) in oven-dried (60 °C) red grape skins compared to freeze-dried samples. Volatile compounds are sensitive to heat because of their polarity and small size, thus evaporate together with moisture (de Torres et al., 2010). Forced-air draft ovens provide constant air velocity and temperatures, while the heat from the sun's ultraviolet radiation fluctuates at different times of the day. Both sun and oven drying methods performed better in reducing the moisture content than freeze-drying, whose performance might be affected by freeze drying condition (low temperatures and pressure).

The higher CP observed for Shiraz, especially when oven-dried, than when sun- or freeze dried could be related to high DM content in oven-dried Shiraz. This could be a plausible explanation considering that freeze drying usually preserves protein content compared to the heat intensity of either oven- and sun-dried samples (Boye et al., 2012). It is important to note that, irrespective of the drying method, red varieties (Shiraz followed by Pinotage) showed higher CP values compared to the white variety (Sauvignon Blanc). This trend was also observed in studies by Baumgärtel et al. (2007) and Basalan et al. (2011). The high CP content in red varieties might be related to the high concentration of proanthocyanidins, which bind to proteins in fermentation grapes during the process of winemaking (Springer et al., 2016), thus may prevent their extraction from the solid residues. The observed CP levels (89.0 – 134 g/ kg DM) across varieties are generally above the normal range for maintenance requirements for low-producing ruminants (60 – 80 g/ kg CP DM) and minimum CP (80 g/ kg DM) required for optimum growth of rumen

microbes (National Research Council, 2007). Irrespective of the drying methods, the present CP values for GP from red varieties were similar to those reported in Europe (89.0 – 155 g/ kg DM) (Baumgärtel et al., 2007; Llobera and Cañellas, 2007; Zalikarenab et al., 2007) and the USA (112 – 123 g/ kg DM) (Deng et al., 2011). Comparable values were reported for the European and Mediterranean white varieties (83.1 – 154 g/ kg DM) (Aslanian et al., 2011; Basalan et al., 2011; Molina-Alcaide et al., 2008) but in present study shows that CP for the white variety was higher compared what has been reported for USA (53.8 - 65.4 g/ kg DM) (Deng et al., 2011) and Brazilian GP varieties (84.9 g/ kg DM) (Sousa et al., 2014).

The findings that most amino acids were in higher concentration in freeze-dried samples regardless of variety could be an indication of their sensitivity to ultraviolet radiation and heat (Boye et al., 2012; Noaman, 2007). The sensitivity depends on various factors (i.e., varieties, processing method and amino acid type) (Boye et al., 2012). Amino acids are easily oxidized or denatured at high temperatures, while freeze drying conditions (low temperature and pressure) assist in maintaining amino acids in their original state (Larrauri et al., 1997; Tseng and Zhao, 2012). In ruminant nutrition, lysine and methionine are frequently limiting amino acids for intensive growth in lambs and methionine for wool production in adult sheep, respectively (Matras et al., 2000; Storm and Ørskov, 1984). The fact holds true for methionine if microbial protein is the only source of protein (Storm and Ørskov, 1984). Nolte et al. (2008) reported that methionine, threonine, arginine, tryptophan and valine limited nitrogen retention in lambs fed a diet low in ruminal undegradable protein.

The observation that methionine was highest in sun-dried Sauvignon Blanc compared to other varieties could be due to varietal or wine processing technique differences. The methionine levels in red varieties might have been reduced by yeast during the fermentation of berries (Bouloumpasi et al., 2002). Compared to the other amino acids, except for glutamine, methionine and cysteine had the lowest content across all GP drying × variety interactions. A similar profile for these sulfur-

containing amino acids was reported by Valiente et al. (1995) for oven-dried GP. Wei et al. (2017) estimated the requirements for methionine and cysteine absorption in Merino sheep maintenance to be between 0.45 – 0.75 g/ day and 0.52 – 0.63 g/ day, respectively, depending on the live weight of the sheep. However, the quantification of the efficiency of amino acid utilization, especially the limiting ones, is complicated by their interconversion (e.g., methionine may be *trans*-sulfurated to cysteine for wool production) and synthesized *de novo* (Storm and Ørskov, 1984; Wei et al., 2017). The present study is an indication that the most limiting amino acids in GP, methionine and cysteine ranging from 0.07 to 0.83 mg/ g DM do not meet the required levels in growing ruminants. Regarding the rest of the amino acids, the majority of them fall short of the range of the requirements for growing ruminant animals (Wilkerson et al., 1993). For instance, Wilkerson et al. (1993) stated that growing beef animal gaining 0.50 g/ kg per day would require 58 and 80 mg/ g of metabolizable histidine and lysine, respectively. In the present study, the highest content of histidine (5.04 mg/ g DM) and lysine (6.56 mg/ g DM) were observed in freeze-dried Pinotage, which is 12 times lower than the required content for both amino acids. Consequently, use of GP in feed requires supplementation of the diet with amino acid or use of high amino acids ingredients to complement the GP.

The observed high starch content reported for Sauvignon Blanc pomace compared to the red varieties, regardless of drying method is in accordance with studies by Deng et al. (2011) and Baumgärtel et al. (2007). Current findings are supported by Corbin et al. (2015) who reported 37.6% (w/ w) water soluble carbohydrates in Sauvignon Blanc GP and 4.6% (w/ w) in Cabernet Sauvignon (red) GP. Baumgärtel et al. (2007) also reported sugars of 39 and 276 g/ kg for red and white GP, respectively. During production of red wine, sugars are fermented by Saccharomyces and non-Saccharomyces yeasts resulting in ethanol biosynthesis (Corbin et al., 2015). In contrast, during production of white wine, grapes are first pressed prior to inoculation with yeast and subsequent fermentation, and therefore retain considerable sugars in the pomace (Corbin et al.,

2015). The low concentration of starch observed for sun- and oven-dried Sauvignon Blanc may be associated with exposure to high temperatures leading to the retrogradation of the amylose macromolecules transforming its linear structure into a crystalline one (Ali et al., 2014). This structural change may have negative implications in the physical properties of starch and the degradation dynamics in the rumen (Ali et al., 2014). The present values for starch (216 – 235 g/ kg DM) are slightly lower than the 230 – 280 g/ kg required for ruminant production (National Research Council, 2007).

High EE or crude fat values for Shiraz pomace, particularly when freeze-dried, could be possibly related to high number of seeds per berry compared to the other varieties (Tesic et al., 2007). The number of seeds per berry are positively correlated to fat content stored in the endosperm (Wen et al., 2016). In addition, the mechanism of freeze-drying enables the preservation of volatile compounds, thus minimize the oxidation of such compounds caused by exposure to ultraviolet radiation or oven temperatures (D'Auria et al., 2009). Overall, the present study has revealed the moderate crude fat (EE) for GP values (49.4 to 106 g/ kg DM) compared to the American varieties (11.4 – 63.3 g/ kg DM) (Deng et al., 2011) and the European varieties, which have large variation (33.0 – 135.3 g/ kg DM) (Llobera and Cañellas, 2007; Winkler et al., 2015). The high EE values reported for Shiraz pomace (90.3 to 105.7 g/ kg DM) may have negative impact on the ruminal fermentation and level of crude fat in the diet should be maintained to below 80 g/ kg DM (Jenkins, 1993). High EE content (>80 g/ kg DM) have been reported to reduce ruminal fiber digestion efficiency because of the toxicity of unsaturated fatty acids (UFA) to rumen microbes (Jenkins, 1993). In addition, high fat content may form a thick deposit around fiber particles, thus reducing the accessibility to microbial digestion (Hur et al., 2017).

The observation that sun-dried Shiraz had the highest aNDFom, ADFom and lignin (sa.) is possibly attributed to effect of radiant heat, which alters the chemical composition of cell soluble components resulting in Maillard complexes, which mimics the physicochemical properties of

lignin (Van Soest, 1994). These findings concur with previous studies which reported higher fiber components for feeds dried between 40 and 65 °C (Bubritt et al., 1988; Dzowela et al., 1995). The high lignin content of GP, especially in red varieties has been attributed to the presence of proanthocyanidins (220 g/ kg DM) and resistant proteins present as insoluble protein– tannin complexes (Llobera and Cañellas, 2007). Aslanian et al. (2011) and Valiente et al. (1995) also reported a similar trend, which was linked to considerable amounts of both cellulose and hemicellulose present in GP. However, some Maillard products are potentially soluble in the neutral detergent solution because they have some acidic tanning properties and are therefore less soluble in acid detergent solution (Terrill et al., 1994). This could be overcome through sequential analysis of NDF followed by ADF on the same samples, especially of proanthocyanidin-containing plant material (i.e., GP) as related to nutrient availability to animals (Pagán et al., 2009).

The period of grape ripening could be another factor that might affect the fiber content in the different varieties, with late maturing red varieties possibly having more fiber than early maturing ones (Jones et al., 2012). This could further be enhanced by the exposure of the berries to the high summer Mediterranean temperatures and ultraviolet radiation as explained earlier. The varietal effect on aNDFom, ADFom and lignin was also noted in both the American and European GP varieties. Deng et al. (2011) reported fiber values ranging from 172 – 281 g/ kg DM (white varieties), with higher values for the red varieties (511 – 563 g/ kg DM). The fiber content of European red and white varieties ranged from 247 – 580 and 282 – 626 g/ kg DM, respectively (Aslanian et al., 2011; Baumgärtel et al., 2007; Zalikarenab et al., 2007). Besides varietal differences, the proportion of GP components could also affect the fiber content, for instance, the inclusion of highly lignified stems (Corbin et al., 2015; Prozil et al., 2012). The American National Research Council recommends a minimum dietary ADF content between 170 and 210 g/ kg DM, and NDF content range of 250 to 330 g/ kg DM for lactating cows (National Research Council, 2001). This makes GP a suitable fiber source for ruminant production through its ability to

stimulate rumination and as a source of energy. A higher fiber content, above what is recommended by the National Research Council (2007) for small ruminants has implications on both intake and digestibility (Allen, 1996).

The observation that sun- and oven- dried, particularly from Shiraz pomace had low fiber digestibility values at 24 and 48 h correspond to the high fiber content reported for these GP drying × variety interactions. This agrees with observations by Abarghuei et al. (2010) who reported low NDF digestibility in sun-dried GP because of its high NDF content (568 g/ kg DM). The high lignin content observed in sun-dried Shiraz lowers digestibility by binding cellulose and hemicellulose, thus preventing degradation of the cell wall by ruminal microbes (Harper and McNeill, 2015). Famuyiwa and Ough (1982) suggested that reduction of lignified material such as grape stalks would improve the digestibility of GP, which could be achieved by efficient destemming of the grape stalks during the harvesting process. Besides the effect of high lignin, proanthocyanidins above the recommended 40 g/ kg DM have been reported to reduce voluntary feed intake and digestibility, subsequently depressing growth in grazing ruminants (Min et al., 2003; Mueller-Harvey, 2006; Waghorn, 2008). However, studies conducted in the tropics (Mlambo et al., 2004; Mueller-Harvey, 2006), have shown that ruminants can tolerate proanthocyanidins of up to 80 g/ kg DM.

The high calcium and phosphorus contents in freeze-dried Shiraz, which was comparable to sun-dried Shiraz is a good indication of the minimal effect sun drying has on these two minerals. This is important considering their importance in livestock production (National Research Council, 2007). It should be noted that the mineral content of GP has wide variations than other chemical components due to the strong influence of the edaphoclimatic conditions, viticultural practices and the winemaking process (García-Lomillo and González-SanJosé, 2017). Overall, the range for calcium (2.29 to 3.73 g/ kg DM) and phosphorus (2.20 to 3.42g/ kg DM) values in the current study were within the recommended range for sheep (1.4 – 7.0 and 0.9 – 3.0 g/ kg DM) and cattle

(2.0 – 11.0 and 1.0 – 3.8 g/ kg DM), respectively (National Research Council, 2007). The mineral profile reflects the typical grape composition reported in literature, with potassium the principal element, being more abundant than calcium, magnesium and sodium (Moncalvo et al., 2016). The high potassium content reported for Pinotage compared to other varieties, irrespective of the drying method, may have emanated from its accumulation in the skin upon the inception of berry ripening, hence its retention in GP (García-Lomillo et al., 2014). Corbin et al. (2015) observed a similar pattern (K > Ca > P) between freeze-dried red and white GP, with the red having 27% more elemental content than its counterpart.

The finding that freeze-dried and oven-dried Pinotage had higher magnesium, sulfur, sodium, iron and aluminum content does not have an immediate explanation. Usually freeze-dried samples are known to retain nutrients better compared to high temperature preservation methods because of its minimal levels of oxidation (Gunya et al., 2016). Mineral content of GP in ruminant feeds is not accurately characterized, most studies have conventionally analyzed separate fractions of pomace (Lachman et al., 2013; Spanghero et al., 2009). However, the lack of differences in the aforementioned minerals is in contrast to a study by Gunya et al. (2016) for *Eisenia foetida* as they reported freeze-dried samples to have higher levels of minerals compared to oven drying. Iron exceeded the 30 – 40 mg/ kg DM required for growing or lactating ruminant animals (National Research Council, 2007) by two- to four-fold across all the drying × variety interactions. The observation that copper content in the Shiraz variety, regardless of the drying method was 30 – 50% higher than other drying × variety interactions could be as a result of its addition through its incorporation as a bio-pesticide in vineyards (Provenzano et al., 2010). Grape pomace could be an important source, considering that copper ranges between 4 – 10 mg/ kg DM in ruminant diets (Gooneratne et al., 1989). It should, however, be noted that sheep are prone to copper poisoning, due to the high sensitivity of the species to mineral, derived mainly from its low capability to conjugate with metallothionein, hence reducing its excretion via bile, and consequently leading to

hepatic accumulation of copper (Reis et al., 2015). On a positive note, the levels of copper in present study were too low to elicit poisoning in sheep since it was below the threshold of 500 mg/g (Reis et al., 2015).

Grape pomace has been recommended as a fiber source to meet maintenance requirements for ruminants (Baumgärtel et al., 2007) but the present study has shown that GP has moderate protein, crude fat and some minerals (potassium, calcium, phosphorus, copper, iron and sulfur), thus makes it a potentially good source of these nutrients for ruminants. Even though the nutrient content had varied across drying × variety interactions, oven-dried Shiraz had favorable DM, ash and CP contents compared to other drying × variety interactions. Similarly, the amino acid and mineral profiles differed with drying × variety interactions but freeze-dried Pinotage had a considerable number of amino acids in high concentration whereas freeze and oven-dried Pinotage drying × variety interactions had more minerals in higher concentration compared to other drying × variety interactions. The study reaffirms that temperatures below 60 °C for nutritive evaluation preserves the integrity of feed samples (Ahn et al., 1997). On a more practical note, sun-drying can be considered a cost-effective method of preservation of GP because of the large quantities produced over a short period of time. A detailed knowledge of GP chemical profile and digestibility enables nutritionists to evaluate its importance as a low-cost alternative feed source for ruminants.

3.5 Conclusions

Overall, oven-dried Shiraz exhibited the parameters with the highest DM, ash and CP, while freeze-dried Sauvignon Blanc had the highest starch content compared to other drying × variety interactions. Freeze-and oven-dried Pinotage had the higher mineral compositions, while freeze-dried Pinotage had a more favorable amino acid profile and ivNDF digestibility at 24 and 48 h than other drying × variety interactions. Further research regarding fatty acid composition, phenolic profile, antioxidant and antimicrobial properties of GP is recommended.

3.6 References

Abarghuei, M.J., Rouzbehan, Y., Alipour, D., 2010. The influence of the grape pomace on the ruminal parameters of sheep. Livest. Sci. 132, 73–79.

Ahn, J.-H., Elliott, R., Norton, B.W., 1997. Oven drying improves the nutritional value of *Calliandra calothyrsus* and *Gliricidia sepium* as supplements for sheep given low-quality straw. J. Sci. Food Agric. 75, 503–510.

Ali, M., Cone, J.W., Hendriks, W.H., Struik, P.C., 2014. Oven-drying reduces ruminal starch degradation in maize kernels. Anim. Feed Sci. Technol. 193, 44–50.

Allen, M.S., 1996. Physical constraints on voluntary intake of forages by ruminants. J. Anim. Sci. 74, 3063–3075.

AOAC (Association of Official Analytical Chemists), 2002. Official Methods of Analysis, 17th ed. Association of Official Analytical Chemists Inc., Arlington, Virginia, USA.

Arvanitoyannis, I.S., Ladas, D., Mavromatis, A., 2006. Potential uses and applications of treated wine waste: A review. Int. J. Food Sci. Technol. 41, 475–487.

Aslanian, A., Dizaji, A.A., Farhoomand, P., Shahryar, H.A., Sis, N.M., Rouhnavaz, S., 2011. Characterization of the nutritive value and protein fractions the Cornell net carbohydrate and protein system in white and red grape (*Vitis vinifera* sp.) pomace. Res. J. Biol. Sci. 6, 298–303.

Barbera, A.C., Maucieri, C., Cavallaro, V., Ioppolo, A., Spagna, G., 2013. Effects of spreading olive mill wastewater on soil properties and crops, a review. Agric. Water Manag. 119, 43–53.

Basalan, M., Gungor, T., Owens, F.N., Yalcinkaya, I., 2011. Nutrient content and in vitro digestibility of Turkish grape pomaces. Anim. Feed Sci. Technol. 169, 194–198.

Baumgärtel, T., Kluth, H., Epperlein, K., Rodehutscord, M., 2007. A note on digestibility and energy value for sheep of different grape pomace. Small Rumin. Res. 67, 302–306.

Beres, C., Costa, G.N.S., Cabezudo, I., da Silva-James, N.K., Teles, A.S.C., Cruz, A.P.G., Mellinger-Silva, C., Tonon, R. V., Cabral, L.M.C., Freitas, S.P., 2017. Towards integral utilization of grape pomace from winemaking process: A review. Waste Manag. 68, 581–594.

Bouloumpasi, E., Soufleros, E.H., Tsarchopoulos, C., Biliaderis, C.G., 2002. Primary amino acid composition and its use in discrimination of Greek red wines with regard to variety and cultivation region. Vitis 41, 195–202.

Boye, J., Wijesinha-Bettoni, R., Burlingame, B., 2012. Protein quality evaluation twenty years after the introduction of the protein digestibility corrected amino acid score method. Br. J. Nutr. 108, S183–S1211.

Brenes, A., Viveros, A., Chamorro, S., Arija, I., 2016. Use of polyphenol-rich grape byproducts in monogastric nutrition. A review. Anim. Feed Sci. Technol. 211, 1–17.

Bubritt, E.A., Pfister, J.A., Malechek, J.C., 1988. Effect of drying method on the nutritive composition of esophageal fistula forage samples: influence of maturity. J. Range Manag. 41, 346–349.

Bustamante, M.A., Moral, R., Paredes, C., Pérez-Espinosa, A., Moreno-Caselles, J., Pérez-Murcia, M.D., 2008. Agrochemical characterisation of the solid byproducts and residues from the winery and distillery industry. Waste Manag. 28, 372–380.

Corbin, K.R., Hsieh, Y.S.Y., Betts, N.S., Byrt, C.S., Henderson, M., Stork, J., DeBolt, S., Fincher, G.B., Burton, R.A., 2015. Grape marc as a source of carbohydrates for bioethanol: Chemical composition, pre-treatment and saccharification. Bioresour. Technol. 193, 76–83.

D'Auria, M., Emanuele, L., Racioppi, R., 2009. The effect of heat and light on the composition of some volatile compounds in wine. Food Chem. 117, 9–14.

De Torres, C., Díaz-Maroto, M.C., Hermosín-Gutiérrez, I., Pérez-Coello, M.S., 2010. Effect of freeze-drying and oven-drying on volatiles and phenolics composition of grape skin. Anal. Chim. Acta 660, 177–182.

Deng, Q., Penner, M.H., Zhao, Y., 2011. Chemical composition of dietary fiber and polyphenols of five different varieties of wine grape pomace skins. Food Res. Int. 44, 2712–2720.

Devesa-Rey, R., Vecino, X., Varela-Alende, J.L., Barral, M.T., Cruz, J.M., Moldes, A.B., 2011. Valorization of winery waste vs. the costs of not recycling. Waste Manag. 31, 2327–2335.

Dwyer, K., Hosseinian, F., Rod, M., 2014. The market potential of grape waste alternatives. J. Food Res. 3, 91-106.

Dzowela, B.H., Hove, L., Mafongoya, P.L., 1995. Effect of drying method on chemical composition and in vitro digestibility of multi-purpose tree and shrub fodders. Trop. Grasslands. 29, 263-269.

El Achkar, J.H., Lendormi, T., Hobaika, Z., Salameh, D., Louka, N., Maroun, R.G., Lanoisellé, J.L., 2016. Anaerobic digestion of grape pomace: Biochemical characterization of the fractions and methane production in batch and continuous digesters. Waste Manag. 50, 275–282.

Famuyiwa, O., Ough, C.S., 1982. Grape pomace: possibilities as animal feed. Am. J. Enol. Vitic. 33, 44–46.

García-Lomillo, J., González-SanJosé, M.L., 2017. Applications of wine pomace in the food industry: approaches and functions. Compr. Rev. Food Sci. Food Saf. 16, 3–22.

García-Lomillo, J., Gonzalez-SanJose, M.L., Del Pino-García, R., Rivero-Perez,´M.D., Muñiz-Rodríguez, P., 2014. Antioxidant and antimicrobial properties of wine byproducts and their potential uses in the food industry. J. Agric. Food Chem. 62, 12595–12602.

Goering, H.K., Van Soest, P.J., 1970. Forage Fiber Analyses (Apparatus, Reagents, Procedures and some Applications) - Agricultural handbook no.379. US Dept. of Agriculture, Washington DC., USA.

Gooneratne, S.R., Buckley, W.T., Christensen, D.A., 1989. Review of copper deficiency and metabolism in ruminants. Can. J. Anim. Sci. 69, 819–845.

Goula, A.M., Thymiatis, K., Kaderides, K., 2016. Valorization of grape pomace: Drying behavior and ultrasound extraction of phenolics. Food Bioprod. Process. 100, 132–144.

Gunya, B., Masika, P.J., Hugo, A., Muchenje, V., 2016. Nutrient composition and fatty acid profiles of oven-dried and freeze-dried earthworm *Eisenia foetida*. J. Food Nutr. Res. 4, 343–348.

Hall, M.B., 2009. Determination of starch, including maltooligosaccharides, in animal feeds: Comparison of methods and a method recommended for AOAC collaborative study. J. AOAC Int. 92, 42–49.

Harper, K., McNeill, D., 2015. The role iNDF in the regulation of feed intake and the importance of its assessment in subtropical ruminant systems (the role of iNDF in the regulation of forage intake). Agriculture 5, 778–790.

Hove, L., Ndlovu, L.R., Sibanda, S., 2003. The effects of drying temperature on chemical composition and nutritive value of some tropical fodder shrubs. Agrofor. Syst. 59, 231–241.

Hur, S.J., Kim, H.S., Bahk, Y.Y., Park, Y., 2017. Overview of conjugated linoleic acid formation and accumulation in animal products. Livest. Sci. 195, 105–111.

Jenkins, T.C., 1993. Lipid Metabolism in the Rumen. J. Dairy Sci. 76, 3851–3863.

Jones, G.V., Reid, R., Vilks, A., 2012. Climate, Grapes, and Wine: Structure and Suitability in a Variable and Changing Climate, in: Dougherty, P.H. (Ed.), The Geography of Wine: Regions, Terrior and Techniques. Spriner Press, pp. 109–133.

Lachman, J., Hejtmánková, A., Hejtmánková, K., Horníčková, Š., Pivec, V., Skala, O., Dědina, M., Přibyl, J., 2013. Towards complex utilisation of winemaking residues: Characterisation of grape seeds by total phenols, tocols and essential elements content as a byproduct of winemaking. Ind. Crops Prod. 49, 445–453.

Larrauri, J.A., Rupérez, P., Saura-Calixto, F., 1997. Effect of drying temperature on the stability of polyphenols and antioxidant activity of red grape pomace peels. J. Agric. Food Chem. 45, 1390–1393.

Llobera, A., Cañellas, J., 2007. Dietary fibre content and antioxidant activity of Manto Negro red grape (*Vitis vinifera*): pomace and stem. Food Chem. 101, 659–666.

Maier, T., Schieber, A., Kammerer, D.R., Carle, R., 2009. Residues of grape (*Vitis vinifera* L.) seed oil production as a valuable source of phenolic antioxidants. Food Chem. 112, 551–559.

Matras, J., Preston, R.L., Bartle, S.J., 2000. Influence of continuous intravenous lysine and methionine infusion on N-balance in growing sheep fed diets that differ in ruminal degradable protein. J. Anim. Feed Sci. 9, 81–89.

Mendes, J.A.S., Xavier, A.M.R.B., Evtuguin, D.V., Lopes, L.P.C., 2013. Integrated utilization of grape skins from white grape pomaces. Ind. Crops Prod. 49, 286–291.

Mertens, D.R., Allen, M., Carmany, J., Clegg, J., Davidowicz, A., Drouches, M., Frank, K., Gambin, D., Garkie, M., Gildemeister, B., Jeffress, D., Jeon, C.S., Jones, D., Kaplan, D., Kim, G.N., Kobata, S., Main, D., Moua, X., Paul, B., Robertson, J., Taysom, D., Thiex, N., Williams, J., Wolf, M., 2002. Gravimetric determination of amylase-treated neutral detergent fiber in feeds with refluxing in beakers or crucibles: Collaborative study. J. AOAC Int. 85, 1217–1240.

Min, B.R., Barry, T.N., Attwood, G.T., McNabb, W.C., 2003. The effect of condensed tannins on the nutrition and health of ruminants fed fresh temperate forages: A review. Anim. Feed Sci. Technol. 106, 3-19.

Mlambo, V., Smith, T., Owen, E., Mould, F.L., Sikosana, J.L.N., Mueller-Harvey, I., 2004. Tanniniferous *Dichrostachys cinerea* fruits do not require detoxification for goat nutrition: *in sacco* and *in vivo* evaluations. Livest. Prod. Sci. 90, 135–144.

Molina-Alcaide, E., Moumen, A., Martín-García, A.I., 2008. Byproducts from viticulture and the wine industry: Potential as sources of nutrients for ruminants. J. Sci. Food Agric. 88, 597–604.

Moncalvo, A., Marinoni, L., Dordoni, R., Garrido, G.D., Lavelli, V., Spigno, G., 2016. Waste grape skins: Evaluation of safety aspects for the production of functional powders and extracts for the food sector. Food Addit. Contam. - Part A Chem. Anal. Control. Expo. Risk Assess. 33, 1116–1126.

Mueller-Harvey, I., 2006. Unravelling the conundrum of tannins in animal nutrition and health. J. Sci. Food Agric. 86, 2010–2037.

National Research Council. 2001. Nutrient Requirements of Dairy Cattle, 7th ed. National Press Academy, Washington, D.C.

National Research Council. 2007. Nutrient Requirements of Small Ruminants. National Academy Press, Washington DC, USA.

Naziri, E., Nenadis, N., Mantzouridou, F.T., Tsimidou, M.Z., 2014. Valorization of the major agrifood industrial byproducts and waste from Central Macedonia (Greece) for the recovery of compounds for food applications. Food Res. Int. 65, 350–358.

Nendel, C., Reuter, S., 2007. Kinetics of net nitrogen mineralization from soil-applied grape residues. Nutr. Cycl. Agroecosystems 79, 233–241.

Noaman, N.H., 2007. Ultraviolet-B irradiation alters amino acids, proteins, fatty acids contents and enzyme activities of *Synechococcus leopoliensis*. Int. J. Bot. 3, 109–113.

Nolte, J.V.E., Löest, C.A., Ferreira, A.V., Waggoner, J.W., Mathis, C.P., 2008. Limiting amino acids for growing lambs fed a diet low in ruminally undegradable protein. J. Anim. Sci. 86, 2627–2641.

Pagán, S., Wolfe, R.M., Terrill, T.H., Muir, J.P., 2009. Effect of drying method and assay methodology on detergent fiber analysis in plants containing condensed tannins. Anim. Feed Sci. Technol. 154, 119–124.

Provenzano, M.R., El Bilali, H., Simeone, V., Baser, N., Mondelli, D., Cesari, G., 2010. Copper contents in grapes and wines from a Mediterranean organic vineyard. Food Chem. 122, 1338–1343.

Prozil, S.O., Evtuguin, D.V., Lopes, L.P.C., 2012. Chemical composition of grape stalks of *Vitis vinifera* L. from red grape pomaces. Ind. Crops Prod. 35, 178–184.

Raffrenato, E., Van Amburgh, M.E., 2011. Technical note: Improved methodology for analyses of acid detergent fiber and acid detergent lignin. J. Dairy Sci. 94, 3613–3617.

Reis, M. de O., Mello, L.S., Cruz, R.A.S. da, Guimarães, L.L., Oliveira, L.G.S. de, Lorenzett, M.P., Pavarini, S.P., Driemeier, D., 2015. Chronic copper toxicity in sheep fed grape marc. Acta Sci. Vet. 43, 108.

Sah, R.N., Miller, R.O., 1992. Spontaneous reaction for acid dissolution of biological tissues in closed vessels. Anal. Chem. 64, 230–233.

SAWIS, 2017. South African Wine Industry Statistics Nr. 42. http://www.sawis.co.za/info/annualpublication.php (accessed 10.18.18)

Sousa, E.C., Uchôa-Thomaz, A.M.A., Osvaldo, J., Carioca, B., De Morais, S.M., De Lima, A., Martins, C.G., Alexandrino, C.D., Augusto, P., Ferreira, T., Livya, A., Rodrigues, M., Rodrigues, S.P., Silva, N., Rodrigues, L.L., 2014. Chemical composition and bioactive

compounds of grape pomace (*Vitis vinifera* L.), Benitaka variety, grown in the semiarid region of Northeast Brazil. Food Sci. Technol. 34, 135–142.

Spanghero, M., Salem, A.Z.M., Robinson, P.H., 2009. Chemical composition, including secondary metabolites, and rumen fermentability of seeds and pulp of Californian (USA) and Italian grape pomaces. Anim. Feed Sci. Technol. 152, 243–255.

Spigno, G., Maggi, L., Amendola, D., Dragoni, M., De Faveri, D.M., 2013. Influence of cultivar on the lignocellulosic fractionation of grape stalks. Ind. Crops Prod. 46, 283–289.

Spigno, G., Marinoni, L., Garrido, G.D., 2017. State of the Art in Grape Processing Byproducts, in: Galanakis, C.M. (Ed.), Handbook of Grape Processing Byproducts: Sustainable Solutions. Academic Press, London, UK, pp. 1–28.

Springer, L.F., Chen, L.A., Stahlecker, A.C., Cousins, P., Sacks, G.L., 2016. Relationship of soluble grape-derived proteins to condensed tannin extractability during red wine fermentation. J. Agric. Food Chem. 64, 8191–8199.

Storm, E., Ørskov, E.R., 1984. The nutritive value of rumen micro-organisms in ruminants. Br. J. Nutr. 52, 613–620.

Terrill, T.H., Windham, W.R., Evans, J.J., Hoveland, C.S., 1994. Effect of drying method and condensed tannin on detergent fiber analysis of *Sericea lespedeza*. J. Sci. Food Agric. 66, 337–343.

Tesic, D., Keller, M., Hutton, R.J., 2007. Influence of vineyard floor management practices on grapevine vegetative growth, yield, and fruit composition. Am. J. Enol. Vitic. 58, 1–11.

Tseng, A., Zhao, Y., 2012. Effect of different drying methods and storage time on the retention of bioactive compounds and antibacterial activity of wine grape pomace (Pinot Noir and Merlot). J. Food Sci. 77.

Valiente, C., Arrigoni, E., Esteban, R.M., Amado, R., 1995. Grape pomace as a potential food fiber. J. Food Sci. 60, 818–820.

Van Soest, P.J., 1994. Nutritional Ecology of the Ruminant. O & B Books, Corvallis, OR, USA.

Waghorn, G., 2008. Beneficial and detrimental effects of dietary condensed tannins for sustainable sheep and goat production-Progress and challenges. Anim. Feed Sci. Technol. 147, 116–139.

Wei, G., Chen, L., Xinmei, G., Fan, Z., Daofu, C., Chenli, L., 2017. Investigation of the postruminal methionine requirement of growing lambs by using the indicator amino acid oxidation technique. Anim. Feed Sci. Technol. 228, 83–90.

Wen, X., Zhu, M., Hu, R., Zhao, J., Chen, Z., Li, J., Ni, Y., 2016. Characterisation of seed oils from different grape cultivars grown in China. J. Food Sci. Technol. 53, 3129–3136.

Wilkerson, V.A., Klopfenstein, T.J., Britton, R.A., Stock, R.A., Miller, P.S., 1993. Metabolizable protein and amino acid requirements of growing cattle. J. Anim. Sci. 71, 2777–2784.

Winkler, A., Weber, F., Ringseis, R., Eder, K., Dusel, G., 2015. Determination of polyphenol and crude nutrient content and nutrient digestibility of dried and ensiled white and red grape pomace cultivars. Arch. Anim. Nutr. 2817, pp.1-14.

Yu, J., Ahmedna, M., 2013. Functional components of grape pomace: Their composition, biological properties and potential applications. Int. J. Food Sci. Technol. 48, 221–237.

Zalikarenab, L., Pirmohammadi, R., Teimuriyansari, A., 2007. Chemical composition and digestibility of dried white and red grape pomace for ruminants. J. Anim. Vet. Adv. 6, 1107–1111.

Chapter 4 Impact of dehydration on retention of bioactive compounds and biological activities of different grape (*Vitis vinifera* L.) pomace varieties[3]

ABSTRACT

The effects of drying method [sun-drying (7 days), oven-drying (72 h at 60 °C) and freeze-drying (~72 h)] and grape (*Vitis vinifera* L. cv. Pinotage, Shiraz and Sauvignon Blanc) variety on pomace fatty acid profile, polyphenolic content and antioxidant capacity were evaluated. Furthermore, the influence of sun-dried pomaces on rumen microbial diversity was assessed *in vitro*. Freeze-dried Shiraz had the highest proportions of 18:1n-9, 18:2n-6, total monounsaturated fatty acid (MUFA), polyunsaturated fatty acids (PUFA) and content of polyphenolics compared to other drying × variety interactions ($P \leq 0.05$). Freeze-dried Sauvignon Blanc had the highest proanthocyanidin content and antioxidant activity for both 2,2-diphenyl-1-picrylhydrazyl (DPPH) and ferric reducing antioxidant power (FRAP) relative to other drying × variety interactions ($P \leq 0.05$). Regardless of variety and inclusion level, grape pomace reduced bacterial species abundance, but

[3] A version of this chapter has been published:

Chikwanha, O.C., Raffrenato, E., Opara, U.L., Fawole, O.A., Setati, M.E., Muchenje, V., Mapiye, C., 2018. Impact of dehydration on retention of bioactive profile and biological activities of different grape (Vitis vinifera L.) pomace varieties. Anim. Feed Sci. Technol. 244, 116–127. doi:10.1016/j.anifeedsci.2018.08.006

improved species diversity, evenness and richness compared to the control ($P \leq 0.05$). Overall, freeze-dried Shiraz had the best fatty acid profile and highest polyphenolic content, while freeze-dried Sauvignon Blanc had the highest proanthocyanidin content and antioxidant activity.

Key words

Antioxidant, Bioactive profile, Dehydration, Grape pomace, Microbial diversity

4.1 Introduction

Grape pomace (GP) is the major byproduct of the wine industry, which equates to 250 g/ kg of the pressed grapes and on a dry basis contains stalks (~20 g/ kg), seeds (~470 g/ kg), skin and pulp (~510 g/ kg) (Beres et al., 2017; Zhang et al., 2017). The wine industry treats GP as waste. Even though GP is not a hazardous waste per se, disposal may be detrimental to the environment due to the phenolic compounds, which decrease the pH of the pomace and increase its resistance to biological degradation (Beres et al., 2017). Generally, GP has low nutritional value because of the high content of phenolic compounds and fiber, particularly lignin (Chapter 3). However, it may be used as ruminant feed because of their well-developed and specialized digestive system that allows better utilization of polyphenolic-rich and fibrous diets (Mlambo and Mapiye, 2015).

Globally, only 3% of GP is currently used as livestock feed, with Australia, a major wine producing country, using 13% as animal feed (Beres et al., 2017; Zhang et al., 2017). From a nutritional point of view, GP is an abundant and inexpensive source of polyphenols that have high antioxidant and antimicrobial properties (García-Lomillo and González-SanJosé, 2017). Furthermore, its lipid fraction presents an interesting fatty acid (FA) profile rich in polyunsaturated FA (PUFA) ranging between 600 – 800 g/ kg of total lipids (Lutterodt et al., 2011; Yi et al., 2009), notably, linoleic acid (18:2n-6) (García-Lomillo and González-SanJosé, 2017). Feeding ruminants diets containing elevated levels of 18:2n-6 and moderate levels of polyphenols (i.e., 20 – 60 g/ kg

proanthocyanidins dry matter; DM) increases tissue accumulation of PUFA and their biohydrogenation intermediate products (Mapiye et al., 2015; Vasta et al., 2010), including rumenic acid [*cis* 9, *trans* 11-18:2, the most abundant conjugated linoleic acid (CLA)] and its precursor vaccenic acid (*trans* 11-18:1), which might have human health benefits (Shokryazdan et al., 2017; Wannamethee et al., 2018). Thus, modulation of ruminal FA metabolism by polyphenols facilitates higher forestomach output of PUFA and their biohydrogenation intermediate products for absorption and incorporation into animal tissues. In that regard, if digested and absorbed GP has the potential to improve meat FA profile and enhance its shelf life through the antioxidative and antibacterial properties of polyphenols.

Fresh GP has high moisture content (500 – 720 g/ kg DM) and if not preserved within a week of pressing, it spoils resulting in pungent odors that attract flies and pests, which transmit pathogenic organisms (Zhang et al., 2017). Overall, high moisture content in feeds and foods is usually associated with elevated water activity, which accelerates microbial spoilage and deteriorative biochemical reactions including oxidation of lipids and degradation of phenolic compounds (Choe and Oh, 2013). Thus, moisture levels in GP should be lowered to preserve it against microbial spoilage and undesirable biochemical reactions.

Dehydration as opposed to fermentation (ensiling) is by far the most common method used to extend the shelf life of GP bioactive compounds for off-season use by decreasing the amount of water available for microbes and deteriorative biochemical reactions (Gan et al., 2017). Dehydration also reduces the bulkiness of GP, which in turn decreases costs of packaging, storage and transportation (Gan et al., 2017). However, some adverse effects on GP quality caused by dehydration including degradation of valuable nutrients (Chapter 3) and loss of bioactive compounds and consequently antioxidant and antimicrobial activities should not be ignored (Gan et al., 2017; Tseng and Zhao, 2012). These losses vary with dehydration technique (Çoklar and Akbulut, 2017; Tseng and Zhao, 2012). Varietal differences among GP are also responsible for

the varying content and composition of bioactive compounds (This et al., 2006), which have a bearing on the resultant biological properties.

In South Africa, GP has relatively been unexploited by the local meat industry as a feed supplement rich in PUFA, natural antioxidants and antibacterials. Overall, GP seasonality is among the main obstacles for its usage as a continuous and steady ingredient in ruminant diets. Furthermore, there is scant literature regarding the effects of dehydration on the retention of bioactive profile and biological activities of different grape (*Vitis vinifera* L.) pomace varieties, particularly the locally bred red variety, Pinotage. The first objective of the current study was to evaluate the effects of drying method and grape variety on pomace FA profile, phenolic composition and antioxidant activity. Secondly, the *in vitro* digestion of sun-dried grape pomaces on ruminal bacterial diversity was investigated.

4.2 Materials and methods

4.2.1 Preparation of grape pomace

The three most commonly produced grape (*Vitis vinifera* L.) varieties (i.e., Pinotage, Sauvignon Blanc and Shiraz) in South Africa were sourced at Stellenbosch University's Welgevallen Experimental farm (Stellenbosch, South Africa). Sauvignon Blanc is a white grape variety, while Pinotage and Shiraz are red varieties. Pinotage was harvested in January, Sauvignon Blanc in February and Shiraz in March 2017. All the varieties were harvested over six consecutive days (6 pressings). Each day, about eight tons of each variety were harvested, pressed and a representative sample (2 kg) of fresh pomace collected (n = 6 pressings). The sample from each day's pressing for each variety was divided into three fractions of 500 g and randomly allocated to three drying treatments: sun-drying for 7 days at temperatures between 25 and 33 °C, oven drying at 60 °C for 72 h and freeze-drying for 72 h (vacuum pressure of 7 mTorr and condenser temperature of -88.7 °C; VirTis Co., Gardiner, NY, USA). The dried samples were ground into

fine powders using a Wiley mill (Model 4, Thomas Scientific, Swedesboro, NJ, USA) with a 1 mm sieve and stored at -20 °C pending analyses.

4.2.2 Extraction of phenolic compounds

The dried GP powders were defatted using n-hexane (10:1; v/ w) with subsequent filtration using a Whatman® # 1 filter. Polyphenols were extracted in acidic acetone [i.e., 70% acetone, 29.9% water and 0.1% hydrochloric acid (v/ v/ v)] according to Tseng and Zhao (2012) in an ultrasonic water bath (Branson B-220H, SmithKline Co., USA) at a solvent to pomace ratio of 10:1 for 20 min at 20 °C. The extracts were centrifuged at 12 857 × g for 15 min at 4 °C and the supernatant recovered and stored at -80 °C pending analyses.

4.2.3 Fatty acid profile of grape pomace

Dried GP (100 mg) was extracted using 5 mL of n-hexane. A 100 µL of 0.1 mL/ L of heptadecanoic acid (17:0) in n-hexane was used as an internal standard before the addition of 1 mL of 2.5% methanolic acid transmethylating reagent. The FA methyl esters were analyzed using Thermo TRACE 1300 series gas chromatography (Thermo Electron S.P.A, Strada Rivoltana, 200090 Rodana, Milan, Italy) with a flame ionization detector using a 30 m TR-FAME capillary column with an internal diameter of 0.25 mm and a 0.25 µm film (Cat. No. HY260M142P, Anatech, Cape Town, South Africa) and a run time of 40 min. The injected volume was 1 µL and the gas chromatography operation conditions were as follows: initial temperature was 50 °C (maintained for 1 min), final temperature of 240 °C. The injector temperature was set at 240 °C and the detector temperature at 250 °C, with hydrogen gas flow rate of 40 mL/ min. The FA methyl esters of each sample was identified by comparing the retention times with those of a standard (Supelco™ 37 Component FA methyl esters mix, Cat no. CRM47885, Supelco, USA). Fatty acids were expressed as g/ 100g of total FA. Analyses were performed in quadruplicate.

4.2.4 Determination of polyphenols and antioxidant activity in grape pomace

Total phenolic and tannin contents were measured by Folin-Ciocalteu colorimetric method (Makkar et al., 2007). A gallic acid standard curve (0.02 – 0.10 mg/ mL) was used and total phenolics and tannins were expressed in grams of gallic acid equivalents per kg DM. Total flavonoid and proanthocyanidin contents were measured using the procedures of Yang et al. (2009) and Porter et al. (1986), respectively. Total flavonoid and proanthocyanidin were expressed as g/ kg DM. Total monomeric anthocyanin concentration was determined using the pH differential method described by Giusti and Wrolstad (2001) and expressed as g/ kg DM. All analyses were performed in triplicate.

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activities of GP extracts were determined according to Karioti et al. (2004). Fifteen µL of extract were mixed with 735 µL of 50% methanol and 750 µL of 0.1 mM of DPPH solution, then incubated in the dark for 30 min. Absorbance was recorded at 517 nm using an ultraviolet-visible spectrophotometer (Thermo Scientific Technologies, Madison, Wisconsin, USA). Ascorbic acid (2 mM) was used as positive control. Radical scavenging activity (RSA, %) = [$Absorbance_{control}$ – ($Absorbance_{sample}$ – $Absorbance_{sample\ background}$)]/ $Absorbance_{control}$ × 100.

The ferric reducing antioxidant power (FRAP) was measured according to the procedure of Benzie and Strain (1996). The FRAP solution contained 25 mL acetate buffer (300 mM acetate buffer, pH 3.6), 2.5 mL of 10 mM of 2,4,6-Tris(2-pyridyl)-*s*-triazine solution, 2.5 mL [20 mM of iron (III) heptahydrate]. Exactly 0.15 mL of extract was mixed with 2.85 mL of FRAP reagent and after 30 min, absorbance was measured at 593 nm. Trolox was used as a standard and FRAP values expressed in µmol Trolox equivalent per g DM. Analyses were performed in triplicate.

4.2.5 Effect of supplementation of sun-dried grape pomace on rumen bacterial diversity

4.2.5.1 Anaerobic digestion of lamb feed supplemented with sun-dried grape pomace

The effect of GP on the rumen bacterial community structure was evaluated for eight sun-dried GP treatments supplemented in lamb finisher diets at 0, 100 and 200 g/ kg inclusion for each grape variety, with two controls (rumen fluid only and feed only). Diets were made isonitrogenous and isoenergetic by substitution of fibrous ingredients with GP (Table 4.1). For the 100 g/ kg GP diet, substitution was mainly for oat bran and wheat bran middlings, and for the 200 g/ kg GP diet the substitution was mainly for maize chop and soybean hulls. Exactly 0.5 g of each test feed was used per treatment. Rumen fluid was collected from two rumen-cannulated Holstein dairy cows fed a total mixed ration *ad libitum*. The diet consisted of lucerne hay (400 g/ kg) and a concentrate (600 g/ kg). Rumen fluid collection was done before the morning feeding into pre-warmed insulated Thermos flasks, mixed thoroughly and filtered through four layers of cheesecloth and glass wool prior to inoculation. Feed samples were mixed with 40 mL of Van Soest buffer in 125 mL Erlenmeyer flasks as described by Goering and Van Soest (1970). The flasks were placed in a heated shaking water bath (39.5 °C) under continuous CO_2 positive pressure to ensure an anaerobic environment, before addition of 10 mL of rumen fluid. Incubation for each sample was conducted over three runs, 2 run replicates and analyses were performed in duplicate. The negative control (buffer + rumen fluid only) was sampled at 0 h and the remainder of the treatments at 24 h. The digesta was frozen at -20 °C pending bacterial community analyses.

4.2.5.2 Chemical analyses of feed and grape pomace

Dry matter (DM; method 934.01), ash (method; 942.05) and ether extract (EE; method 920.39) contents were determined according to the AOAC (2002) procedures. Total nitrogen content was

analyzed using the Dumas method with a macro-Nitrogen analyzer (LECO® FP528, LECO Corporation, Miami, USA). Crude protein (CP) was calculated by multiplying the nitrogen content by a factor of 6.25. Starch was measured using a commercial assay (Total Starch Megazyme kit KTSTA, Megazyme International Ireland Ltd., Wicklow, Ireland), following the method for samples containing glucose and/ or maltodextrins (Hall, 2009). Neutral detergent fiber (aNDFom) was determined using heat-stable alpha-amylase and addition of sodium sulfite (Mertens, 2002). Acid detergent fiber (ADFom) analysis was performed according to AOAC (method 962.09; 2002). Lignin (sa.) was analyzed according to Goering and Van Soest (1970) as modified by Raffrenato and Van Amburgh (2011). Neutral detergent fiber, ADFom and lignin (sa.) were expressed exclusive of ash. Analyses of feed and grape pomace were performed in triplicate. Total tannin and proanthocyanidin contents were analyzed using same methods as described in Section 4.2.4.

4.2.5.3 DNA extraction and ARISA Fingerprinting

After 24 h incubation period, 200 µL of the rumen fluid mixture was sampled for genomic bacterial DNA extraction according to the manufacturers' specifications (FavorPrep™ Soil DNA Isolation Mini Kit, Favorgen® Biotech Corporation, Taiwan). The bacterial communities were evaluated by amplifying the intergenic transcribed spacer (ITS) region (i.e., located between the 16S and 23S rRNA genes) with the 6FAM-ITSF (5′-GTCGTAACAAGGTAGCCGTA-3′) and ITS-Reub (5′-GCCAAGGCATCCACC-3′) primer pair (Cardinale et al., 2004). Automated Ribosomal Intergenic Spacer Analysis (ARISA) was performed to assess the variability of bacterial community between treatments. The ARISA data was processed using GeneMarker® V2.4.0 (Soft Genetics) and then imported onto the T-REX (T-RFLP Analysis Expedited) platform (Culman et al., 2009) where the peaks were filtered and aligned. Subsequently, a data matrix was

generated and used for further analyses. Polymerase chain reaction and the ARISA technique were performed as described by Setati et al. (2012) in duplicate.

Table 4.1 Feed ingredients and chemical composition of experimental diets (g/ kg DM)

Item	Inclusion of grape pomace (g/kg)			
	0	100	200	
Ingredients				
Sun-dried Pinotage meal[1]	0	100	200	
Lucerne meal	200	200	200	
Soybean hulls	37.6	48.2	5.6	
Hominy chop (maize bran)	50	50	0	
Defatted maize germ	150	150	130	
Wheat bran middlings	90.9	46.8	0	
Oat bran	50	0	0	
Maize meal	281	292	325	
Megalac[2]	0	0	20.7	
Molasses syrup	40	40	40	
Fish meal[3]	0	0	3.81	
Lupins	13.3	5.97	0	
Soybean oil cake[4]	38.7	33.3	46.9	
Limestone fine	24.8	18.8	13.3	
Salt fine	8.2	4.4	4.2	
Toxin binder (Mycosorb A)	1	1	1	
Mold inhibitor (Technigard)	0.5	0.5	0.5	
Sal CURB® S liquid mix[5]	5	0	0	
Vitamin/Mineral Premix[6]	7	7	7	
Dust binder (Dustex)	2	2	2	
Chemical composition				SEM[12]
DM (g/ kg as-fed basis)	880	878	881	0.55
Ash	89.7	71.8	75.6	1.05
Organic matter	910	928	924	1.05
Crude protein	173	179	174	1.95
Ether extract	37.1	49.8	63.4	0.90
Starch	285	283	284	2.12
aNDFom[7]	300	289	243	6.27
ADFom[8]	190	166	182	3.77
Lignin (sa.) [9]	24.3	47.0	60.3	2.10
Metabolizable Energy (MJ/ kg DM)[10]	10.9	11.6	11.4	0.08
Non-fiber carbohydrates[11]	401	410	444	6.43
Total tannins (g gallic acid equivalent/ kg DM)	0	34.7	59.4	2.06
Proanthocyanidins (g cyanidin chloride equivalent/ kg DM)	0	12.3	25.7	0.24

[1] Chemical composition GP meal: 919 g/ kg DM; ash, 56.8 g/ kg; crude protein, 107 g/ kg DM; ether extract, 89.3 g/ kg DM; starch, 69.2 g/ kg DM, aNDFom, 401 g/ kg; total tannins, 143 g/ kg DM; proanthocyanidins, 64.9 g/ kg DM.
[2] Megalac: A high-energy rumen-protected fat supplement (calcium salt of palm fatty acids)
[3] Fish meal containing 650 g/ kg protein.
[4] Soybean oil cake: Soybean containing 470 g/ kg protein.
[5] Sal CURB® S liquid mix: antimicrobial used to control *Salmonella* contamination.
[6] Vitamin/ mineral premix (i.e., MW Sheep PX with Monesin 68813). The composition of the vitamin/ premix not included because of an agreement by the feed manufacturer not to disclose confidential information.
[7] aNDFom: neutral detergent fiber assayed with heat stable amylase and expressed exclusive of ash.
[8] ADFom: Acid detergent fiber expressed exclusive of ash.
[9] Lignin (sa): Lignin determined by solubilization of cellulose with sulfuric acid.
[10] Estimated according to CSIRO (2007).
[11] Non fiber carbohydrates: Calculated as: 1000– (aNDFom g/ kg + crude protein g/ kg + ether extract g/ kg + ash g/ kg).
[12] SEM: Standard error of means.
Number of samples analyzed per treatment = 4. Chemical analyses of samples were done in triplicate

4.2.6 Statistical analyses

Data for FA profile, phenolic composition and antioxidant activity were analyzed as a completely randomized design with a 3 × 3 factorial arrangement of treatments using the generalized linear model procedure of SAS (2012). The total number of observations for phenolic content and antioxidant activity data were 162 (3 drying methods × 3 varieties × 6 pressings × 3 replications). Similarly, the total number of observations for fatty acids were 216 because it had four replications. After averaging the replications, the remaining 54 observations were subjected to analysis of variance using the following model:

$$Y_{ijk} = \mu + D_i + V_j + (DV)_{ij} + \varepsilon_{ijk},$$

Where:

Y_{ijk} = FA composition, phenolic content, antioxidant activity,

μ = overall mean,

D_i= effect of i^{th} drying method (i^{th} = sun, oven, freeze),

V_j = effect of j^{th} variety (j = Pinotage, Sauvignon Blanc, Shiraz),

$(DV)_{ij}$= interaction of i^{th} drying method and j^{th} variety and

ε_{ijk} = residual error.

The generalized linear model procedure of SAS (2012) was also used for the microbial diversity with variety as the main fixed effect. The total number of observations used were 96 (8 treatments × 3 runs × 2 run replicates × 2 duplications for microbial diversity analyses). On averaging run replicates, the remaining 48 observations were subjected to analysis of variance using the following model:

$$Y_{ij} = \mu + T_i + \varepsilon_{ij},$$

Where,

Y_{ij} = species abundance, species diversity (Shannon index), Buzas and Gibson's evenness ($e^{\wedge H/S}$) and Chao1 (richness),

μ = overall mean,

T_i = effect of i^{th} treatment (i^{th} = rumen fluid only, feed only, Pinotage100, Pinotage200, Sauvignon Blanc100, Sauvignon Blanc200, Shiraz100, Shiraz200) and,

ε_{ij} = residual error.

For FA composition, phenolic content, antioxidant activity and microbial diversity statistical models, the PDIFF option adjusted by the Tukey method was included in the LSMEANS statement to account for multiple comparisons among treatments. Differences between treatments were declared significant at $P \leq 0.05$.

Principal component analysis (PCA) was carried out using XLSTAT software version 19.4 (Addinsoft, France) to determine the pattern of inter-correlations among phenolics with antioxidant and bacterial diversity (variables) and variability of samples in relation to GP variety and drying method sampled. Diversity analyses and statistical comparison of the microbial communities were performed in PAleontological STatistics software (Hammer et al., 2001).

4.3 Results

4.3.1 Grape pomace fatty acid profile

The interactive effects for drying method and variety had significant effects on total FA content and all the FAs identified ($P \leq 0.05$; Table 4.2). Overall, Shiraz had higher ($P \leq 0.05$) total FA content than other varieties, with the freeze-dried one having the highest content followed by sun-dried and oven-dried ones, respectively ($P \leq 0.05$). Linoleic acid (18:2n-6; 57.7 – 72.7 g/ 100g of total FA) was the most dominant FA followed by palmitic acid (16:0; 6.7 – 15.8 g/ 100g of total FA), oleic acid (18:1n-9; 7.7 – 12.3 g/ 100g of FA), stearic acid (18:0; 4.2 – 7.2 g/ 100g of total FA), and α-linolenic acid (18:3n-3; 1.5 – 2.7 g/ 100g of total FA) in that order ($P \leq 0.05$).

Sauvignon Blanc had the highest percentage of 16:0, 18:0 and total SFA in ascending order of freeze-dried, oven-dried and sun-dried ($P \leq 0.05$; Table 4.2). The proportions of 18:1n-9 and total monounsaturated FA (MUFA) were highest for freeze-dried Shiraz compared to other drying × variety interactions ($P \leq 0.05$; Table 4.2). Linoleic acid was the most dominant PUFA contributing more than 60% of the total PUFA. Freeze-dried Shiraz had the highest percentage of 18:2n-6 and total n-6 PUFA followed by freeze-dried Pinotage and sun-dried Shiraz, respectively ($P \leq 0.05$). Freeze-dried Sauvignon Blanc had the highest proportions of 18:3n-3 followed by sun-dried Shiraz ($P \leq 0.05$). However, regarding total n-3 PUFA, sun-dried Sauvignon Blanc and freeze-dried Shiraz had the highest proportions, followed by sun-dried Sauvignon Blanc ($P \leq 0.05$).

Table 4.2 Effects of drying method, grape pomace variety and their interactions on FA composition (g/ 100g of total FA) and total fat content (g/ 100g DM)

Fatty acid	Sun drying			Oven drying			Freeze drying			SEM‡	P values		
	Pinotage	*S. Blanc	Shiraz	Pinotage	*S. Blanc	Shiraz	Pinotage	*S. Blanc	Shiraz		D	V	D × V
10:0	0.27^{a}	0.21^{c}	0.11^{de}	0.25^{ab}	0.03^{f}	0.13^{d}	0.24^{b}	0.02^{f}	0.10^{e}	0.012	0.01	<0.01	<0.01
12:0	0.37^{a}	0.22^{c}	0.27^{b}	0.35^{a}	0.12^{d}	0.21^{c}	0.25^{b}	0.06^{e}	0.13^{d}	0.008	<0.01	<0.01	0.01
14:0	0.34^{d}	0.46^{a}	0.37^{c}	0.33^{d}	0.41^{b}	0.30^{e}	0.16^{g}	0.25^{f}	0.15^{g}	0.007	<0.01	<0.01	<0.01
15:0	0.39^{b}	0.44^{a}	0.27^{d}	0.36^{c}	0.21^{e}	0.26^{d}	0.20^{e}	0.15^{f}	0.16^{f}	0.013	<0.01	<0.01	<0.01
16:0	10.8^{e}	15.8^{a}	10.6^{f}	12.3^{d}	14.6^{b}	12.4^{d}	8.90^{g}	13.3^{c}	6.70^{h}	0.02	<0.01	<0.01	<0.01
18:0	6.49^{d}	7.17^{a}	4.45^{h}	5.93^{e}	6.54^{b}	4.74^{g}	4.95^{f}	6.82^{c}	4.17^{i}	0.021	<0.01	<0.01	0.01
20:0	0.40^{c}	0.53^{a}	0.33^{e}	0.35^{de}	0.45^{b}	0.35^{de}	0.21^{g}	0.26^{f}	0.17^{h}	0.006	<0.01	0.003	0.01
22:0	0.59^{b}	0.32^{h}	0.39^{f}	0.44^{e}	0.65^{a}	0.56^{c}	0.26^{g}	0.47^{d}	0.25^{g}	0.008	<0.01	<0.01	<0.01
24:0	0.57^{e}	0.82^{d}	1.38^{a}	0.53^{f}	1.33^{b}	0.53^{f}	0.28^{g}	1.03^{c}	0.26^{g}	0.013	<0.01	<0.01	<0.01
∑ SFA	20.8^{e}	26.0^{a}	16.1^{g}	21.5^{d}	24.8^{b}	19.9^{f}	16.0^{g}	23.5^{c}	11.4^{h}	0.09	<0.01	<0.01	<0.01
16:1	0.80^{c}	0.16^{h}	0.18^{h}	1.06^{b}	1.17^{a}	0.57^{d}	0.44^{e}	0.39^{f}	0.29^{g}	0.011	<0.01	<0.01	<0.01
18:1n-9	11.1^{c}	12.2^{b}	8.60^{f}	10.1^{d}	7.68^{h}	11.1^{c}	9.18^{e}	8.27^{g}	12.3^{a}	0.02	<0.01	<0.01	<0.01
∑ MUFA	12.1^{c}	12.5^{b}	8.91^{g}	11.3^{e}	12.1^{c}	11.8^{d}	9.70^{f}	8.79^{h}	12.6^{a}	0.022	<0.01	<0.01	<0.01
18:2n-6	63.9^{e}	57.7^{g}	69.3^{c}	64.7^{d}	63.5^{e}	64.8^{d}	71.7^{b}	62.5^{f}	72.7^{a}	0.15	<0.01	<0.01	<0.01
20:2n-6	0.12^{a}	0.09^{b}	0.10^{b}	0.09^{b}	0.13^{a}	0.09^{b}	0.06^{c}	0.10^{b}	0.05^{c}	0.006	<0.01	<0.01	<0.01
22:2n-6	0.23^{d}	0.31^{b}	0.17^{ef}	0.31^{b}	0.46^{a}	0.33^{b}	0.15^{f}	0.26^{c}	0.19^{e}	0.011	<0.01	<0.01	<0.45
∑n-6	64.6^{e}	58.1^{g}	69.6^{c}	65.4^{d}	64.3^{ef}	65.9^{d}	72.3^{b}	64.0^{f}	73.8^{a}	0.15	<0.01	<0.01	<0.01
18:3n-3	1.97^{d}	2.19^{c}	2.52^{b}	1.50^{f}	1.54^{f}	2.21^{c}	1.90^{e}	1.99^{d}	2.66^{a}	0.024	<0.01	<0.01	<0.01
20:3n-3	0.03^{e}	0.19^{b}	0.20^{b}	0.10^{d}	0.19^{b}	0.13^{e}	0.04^{e}	0.27^{a}	0.10^{d}	0.009	0.45	<0.01	<0.01
22:6n-3	0.06^{d}	0.09^{a}	0.08^{b}	0.05^{e}	0.07^{c}	0.03^{f}	0.03^{f}	0.06^{d}	0.02^{g}	0.037	<0.01	<0.01	0.04
∑n-3	2.06^{e}	2.46^{b}	2.79^{a}	1.64^{h}	1.79^{g}	2.38^{c}	1.97^{f}	2.32^{d}	2.78^{a}	0.018	0.01	0.01	<0.01
n-6: n-3	31.3^{d}	23.6^{h}	24.9^{g}	39.9^{a}	35.9^{c}	27.7^{e}	36.7^{b}	27.6^{e}	26.6^{f}	0.24	<0.01	<0.01	<0.01
∑PUFA	66.6^{ef}	60.6^{h}	72.4^{c}	67.0^{e}	66.1^{g}	68.2^{d}	74.2^{b}	66.3^{fg}	76.6^{a}	0.16	<0.01	<0.01	<0.01
P: S	3.20^{e}	2.33^{i}	3.99^{c}	3.13^{f}	2.67^{h}	3.43^{d}	4.61^{b}	2.82^{g}	6.71^{a}	0.020	<0.01	<0.01	<0.01
Total fat	6.88^{e}	5.28^{f}	9.52^{b}	6.79^{e}	4.99^{g}	9.03^{c}	7.72^{d}	4.94^{g}	10.6^{a}	0.652	<0.01	<0.01	<0.01

Least square means with different superscript within a row are significantly different ($P \leq 0.05$).
*S. Blanc - Sauvignon Blanc; SFA- saturated fatty acids; MUFA- monounsaturated fatty acids; PUFA- polyunsaturated fatty acids; n-6 –omega PUFA 6; n-3 –omega 3 PUFA; P: S, polyunsaturated fatty acids to saturated fatty acids ratio.
Number of samples analyzed per variety for each drying method = 6. Chemical analyses were done in quadruplicate.
‡SEM, standard error of means.

4.3.2 Grape pomace phenolic content and antioxidant activity

Phenolic contents were significantly influenced by the drying × variety interaction ($P \leq 0.05$; Table 4.3). Across interactions, freeze-dried Shiraz had the highest contents of total phenols, tannins, flavonoids and anthocyanins followed by freeze-dried Pinotage and sun-dried Shiraz in that order ($P \leq 0.05$), except for anthocyanin contents, which were higher ($P \leq 0.05$) for oven-dried Shiraz compared to sun-dried Shiraz (Table 4.3). Overall, the freeze-drying method had higher ($P \leq 0.05$) proanthocyanidin contents compared to other drying methods, with Sauvignon Blanc variety having the highest value followed by Shiraz and Pinotage, respectively ($P \leq 0.05$).

Drying method, grape variety and their interaction had significant effects on DPPH and FRAP antioxidant assays ($P \leq 0.05$; Table 4.3). In the case of the DPPH assay, the radical scavenging activity (RSA %) for freeze-dried Sauvignon Blanc and sun-dried Shiraz had higher ($P \leq 0.05$) values compared to other drying × variety interactions, with no significant difference between the two ($P > 0.05$). Intermediate RSA% were observed for both sun-dried Shiraz and Sauvignon Blanc ($P \leq 0.05$), with no significant difference between them ($P > 0.05$). The lowest RSA was observed for sun-dried Pinotage and oven-dried Sauvignon Blanc ($P \leq 0.05$). However, RSA for all drying × variety interactions were lower ($P \leq 0.05$) compared to the positive control (2mM ascorbic acid) at 85.9%. The pomace extracts examined in the present study also exhibited significant FRAP values ranging between 45.1 and 50.7 µmol Trolox equivalent / g DM. Freeze-dried Pinotage had the least reducing power compared to the other freeze-dried varieties ($P \leq 0.05$).

The relationship among dried grape pomaces, phenolics and antioxidant potential was assessed by the PCA, with the first and second principal components accounting for 64.2 and 20.2% of the total variation, respectively (Fig. 4.1). From principal component 1, freeze-dried Pinotage and Shiraz were clustered together with total phenolics, total tannins and anthocyanins in the lower-right quadrant. On the other hand, freeze-dried Sauvignon Blanc was closely clustered

in the upper-right quadrant with DPPH and proanthocyanidins. The rest of the drying × variety interactions were clustered in the lower- and upper-left quadrants of PCA (Fig. 4.1).

Table 4.3 Effects of drying method, grape pomace variety and their interactions on phenolic composition (g/ kg DM)

Item	Sun drying			Oven drying			Freeze drying			SEM‡	P values		
	Pinotage	*S. Blanc	Shiraz	Pinotage	*S. Blanc	Shiraz	Pinotage	*S. Blanc	Shiraz		D	V	D × V
TPC[1]	110de	87.7^{f}	135^{c}	116^{d}	78.8^{g}	114de	182^{b}	108^{e}	206^{a}	2.82	<0.01	<0.01	<0.01
Total tannins[1]	88.8^{d}	74.8^{e}	115^{c}	97.3^{d}	66.1^{e}	91.9^{d}	154^{b}	96.7^{d}	175^{a}	3.19	<0.01	<0.01	<0.01
Proanthocyanidins[2]	59.5^{e}	60.2^{e}	63.5^{d}	54.9^{f}	64.2^{d}	60.9^{e}	72.0^{c}	87.0^{a}	77.3^{b}	1.14	<0.01	<0.01	<0.01
Total flavonoids[3]	107^{e}	90.6^{f}	153^{c}	119^{e}	66.8^{g}	139^{d}	166^{b}	132^{d}	187^{a}	4.27	<0.01	<0.01	0.01
Anthocyanins[4]	0.07^{f}	0.07^{f}	1.30^{d}	0.95^{e}	0.04^{f}	1.78^{c}	1.91^{b}	0.07^{f}	2.92^{a}	0.055	<0.01	<0.01	<0.01
DPPH[5]	61.8^{e}	78.9^{b}	81.4^{ab}	67.8^{d}	60.8^{e}	71.4^{c}	71.9^{c}	83.4^{a}	70.9^{cd}	1.96	<0.01	0.01	<0.01
FRAP[6]	48.0^{d}	45.1^{f}	48.8^{c}	48.2^{cd}	46.9^{e}	47.0^{e}	49.7^{b}	50.7^{a}	50.1^{ab}	0.27	<0.01	<0.01	<0.01

Least square means with different superscript within a row are significantly different ($P \leq 0.05$).
*S. Blanc, Sauvignon Blanc.
‡SEM, standard error of means.
[1] TPC, Total phenolic content and total tannins expressed as gallic acid equivalent.
[2] Proanthocyanidin content expressed as cyanidin chloride equivalent.
[3] Total flavonoid content expressed as catechin equivalent.
[4] Anthocyanins expressed as cyanidin-3-glucoside equivalent.
[5] DPPH, 2,2-diphenyl-1-picrylhydrazyl expressed as % radical scavenging activity.
[6] FRAP, Ferric reducing antioxidant potential expressed as µM Trolox equivalent per gram DM.
Number of samples analyzed per variety for each drying method = 6. Chemical analyses were done in triplicate.

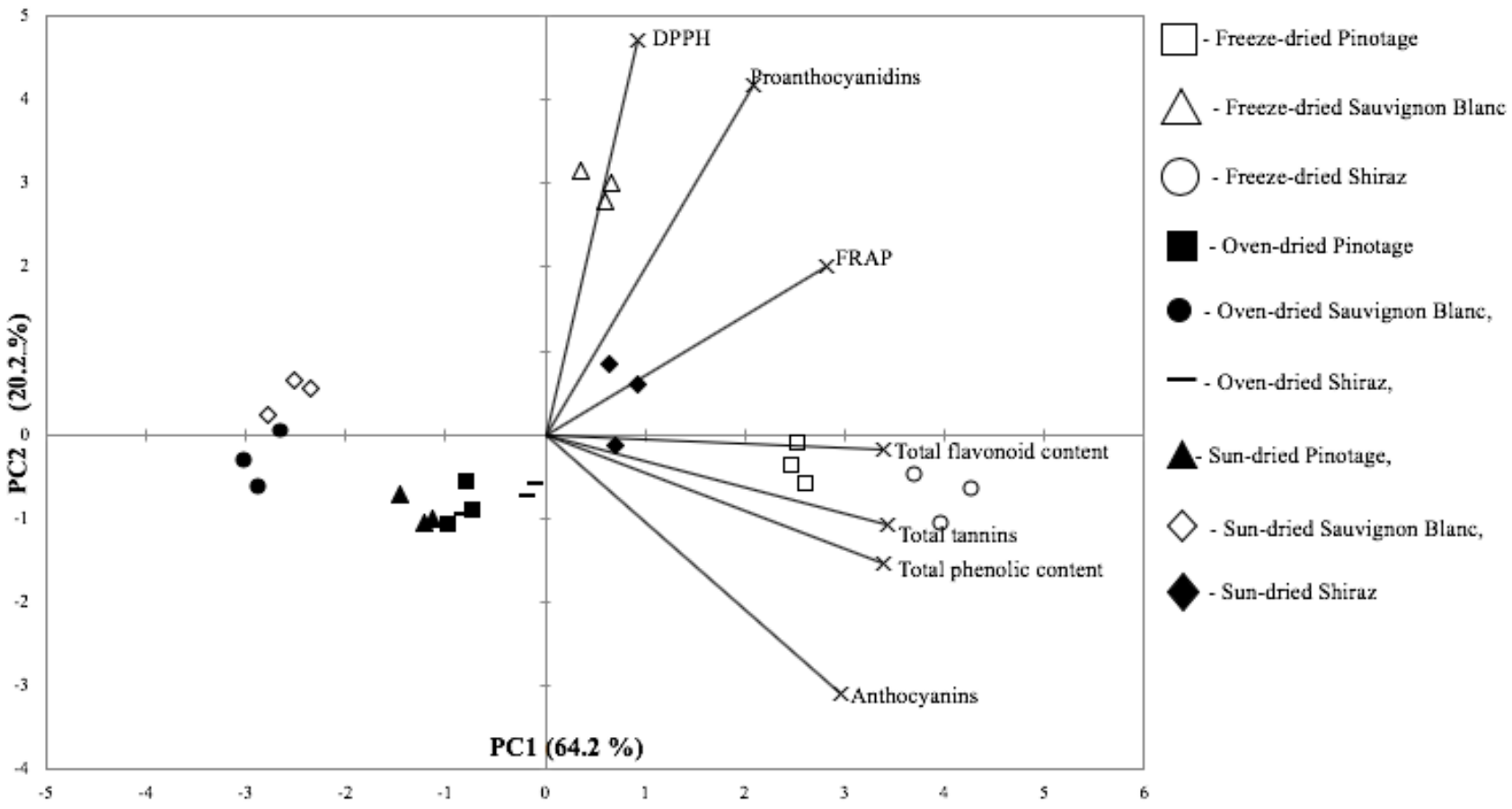


Fig. 4.1 Principle component analysis plot describing the relationship of bioactive parameters and drying method × grape pomace variety interactions.

4.3.3 Influence of sun-dried grape pomace on rumen microbiota

Grape pomace variety was significant ($P \leq 0.05$; Table 4.4) for all microbial parameters evaluated. Bacterial species abundance in the rumen fluid was higher ($P \leq 0.05$) than in GP treatments. Overall, bacterial diversity (Shannon index) and Buzas and Gibson's evenness ($e^{\wedge H/S}$) were higher ($P \leq 0.05$) in the GP treatments compared to the rumen fluid. No differences were observed in the richness (Chao 1 index) in all treatments with lamb feed (Table 4.4). The first and second principal components accounted for 90.2 and 6.40% of the total variation, respectively (Fig. 4.2). The PCA was able to separate the ruminal communities of the negative control (rumen fluid only) from the rest of the treatments. The analysis of similarity (ANOSIM) between and within groups was high (R-ANOSIM; 0.69). Based on ANOSIM the majority of the groups were different ($0.5 < R \leq 0.75$) to highly different ($0.75 < R \leq 1$) except for Pinotage and feed ($R = 0.32$; different but with some overlap), and Pinotage and Shiraz ($R = 0.21$; similar with some differences).

Table 4.4 *In vitro* digestion of lamb finisher diets supplemented with three sun-dried grape pomace varieties on ruminal bacterial diversity

Item	Treatments								SEM‡	P value
	Rumen fluid	Lamb feed	Pinotage 100[1]	Pinotage 200[2]	*S. Blanc 100[1]	*S. Blanc 200[2]	Shiraz 100[1]	Shiraz 200[2]		
Species abundance	84.0[a]	69.0[bc]	63.5[c]	63.0[c]	69.5[b]	63.0[c]	69.0[b]	66.5[bc]	2.02	0.01
Species diversity	2.25[d]	3.66[bc]	3.82[a]	3.89[a]	3.58[c]	3.82[a]	3.79[a]	3.74[ab]	0.08	<0.01
Evenness	0.29[d]	0.59[b]	0.63[ab]	0.67[a]	0.62[b]	0.63[ab]	0.66[a]	0.62[b]	0.02	<0.01
Species richness	32.5[b]	66.5[a]	72.5[a]	72.5[a]	68.0[a]	72.5[a]	66.5[a]	68.5[a]	3.02	0.01

Least square means with different superscript within a row are significantly different ($P \leq 0.05$).
*S. Blanc- Sauvignon Blanc.
‡SEM, standard error of means.
[1] 100 g/ kg grape pomace inclusion.
[2] 200 g/ kg grape pomace inclusion.
Number of samples analyzed per run = 3. Chemical analyses for run replicates were done in duplicate.

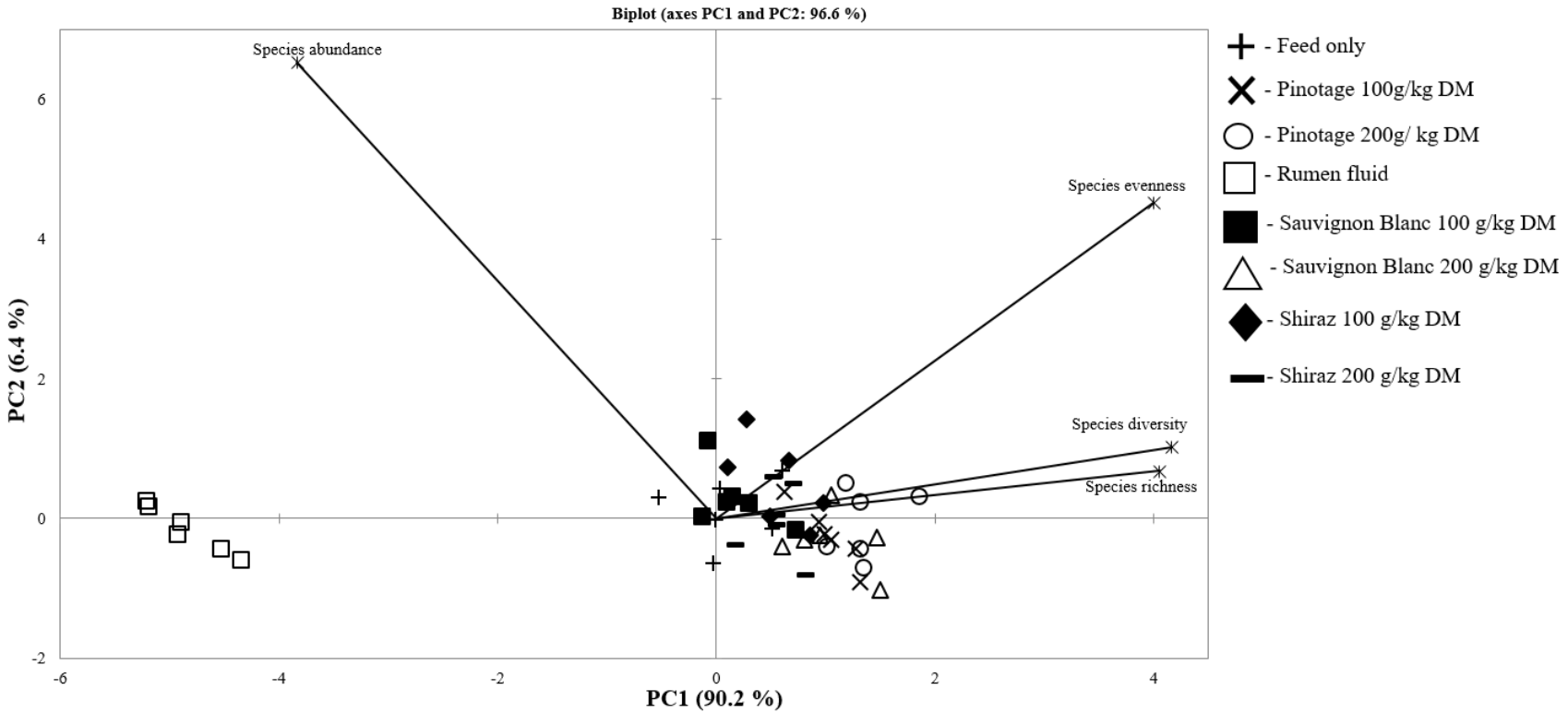


Fig. 4.2 Principle component analysis of describing the effect sun-dried grape pomaces (g/ kg DM) in lamb finisher diets on ruminal bacterial community changes *in vitro*.

4.4 Discussion

The higher fat content values for Shiraz variety, irrespective of the drying method were related to the variety and possibly the high number of seeds per berry as reported in Chapter 3. The higher proportions of individual and total SFA for sun-dried Sauvignon Blanc might be attributed to their stability due to the absence of double bonds, coupled with the protective effects conferred by phenolic antioxidants in GP. Overall, the proportions of 16:0 and 18:0 were comparable to previous literature (7.1 – 18.2 and 2.4 – 5.5 g/ 100 g of total FA, respectively; Al Juhaimi et al., 2017; Lutterodt et al., 2011; Yi et al., 2009).

The high proportions of 18:1n-9 and total MUFA observed for freeze-dried Shiraz might be associated with drying conditions (Çoklar and Akbulut, 2017), while the variability among the varieties can be explained by genetic factors (Sabir et al., 2012). Freeze drying enhances the stability and preservation of heat labile bioactive compounds such as unsaturated FA (Çoklar and Akbulut, 2017; Tseng and Zhao, 2012). The observation that 18:2n-6 was the most abundant FA is consistent with previous studies (Hixson et al., 2016; Lutterodt et al., 2011; Yi et al., 2009). Although this may be explained by genetic factors (Sabir et al., 2012; This et al., 2006), it is well accepted that environmental conditions have considerable effects on the FA composition of plants (Sabir et al., 2012). Meï et al. (2015) found a positive association between PUFA proportions and growth rate in plant cell cultures; where the growth rate is a function of genetic influences (This et al., 2006) and environmental factors including fertilizer application (Jackson and Lombard, 1993). Early harvesting reduced the 18:2n-6 proportions for Razaki (i.e., a red table grape variety), while there was moderate increment for Cardinal (i.e., a white table grape variety), indicating interactive effects of varietal and environmental conditions (Çoklar and Akbulut, 2017). The

significance of feeding linoleic acid-rich feeds to ruminants is their potential to increase the duodenal flow of 18:2n-6 and its biohydrogenation intermediate products such as rumenic acid and vaccenic acid among other CLA and *trans*-18:1 isomer (Mapiye et al., 2012).

Similar to 18:2n-6, the high proportions of 18:3n-3 for freeze-dried Sauvignon Blanc may be attributed to varietal differences and mild nature (i.e., low temperature and pressure) of freeze drying. The current values for 18:3n-3 are comparable to those reported by Hixson et al. (2016) ranging between 0.3 to 13.5 g/ kg of total FA. Furthermore, high PUFA levels across treatments are also in agreement with studies by Yi et al. (2009) and others (Lutterodt et al., 2011; Wen et al., 2016). Overall, the lower PUFA values for the sun- and oven-dried varieties compared to the freeze-dried varieties could have been caused by the slow drying rate and 60 °C temperature for sun- and oven-drying methods, respectively (Çoklar and Akbulut, 2017). This relates to the susceptibility of PUFA double bonds to oxidation (Lutterodt et al., 2011). However, for the current study, sun-dried Sauvignon Blanc had the highest total n-3 PUFA, an anomaly that could be related to the presence of flavonoids, especially proanthocyanidins and the effect of their protective chromophoric ring structure which absorbs light in both the ultraviolet and visible spectra thus conferring stability of such susceptible compounds from ultraviolet radiation (Downey et al., 2006). This explanation is further supported by the findings by Lutterodt et al. (2011) who reported no positive relationship between low oxidative stability index and low PUFA proportions for grape seed oil. Although, 18:3n-3 was detected in low percentages, its potency is relevant in ruminant nutrition as a precursor of long-chain n-3 PUFA and the associated benefits to human health (Shokryazdan et al., 2017; Wannamethee et al., 2018). Unlike grape seed oil, there is scant

literature on the FA profile of GP powder, which makes comparison of the two products challenging.

The higher contents of total phenolics, tannins, flavonoids and anthocyanins for freeze-dried Shiraz and proanthocyanidins for freeze-dried Sauvignon Blanc compare to other drying × variety interactions could be dependent on the genotype of the cultivar (This et al., 2006), ontogeny (i.e., berry development and maturation (Downey et al., 2006) and drying method (Tseng and Zhao, 2012). Similar findings were also reported by Çoklar and Akbulut (2017) in grapes and winery byproducts (Larrauri et al., 1997; Tseng and Zhao, 2012). Overall, the decrease of phenolic compounds for the sun- and oven-dried varieties in Table 4.2 is further supported by the PCA results (Fig. 4.1), where the thermally treated samples were clustered in the left quadrants while phenolic compounds were clustered in the right quadrants. This could be credited to the thermal degradation and/ or oxidation of these compounds, especially to due to prolonged heat exposure, which can cause irreversible chemical changes (Çoklar and Akbulut, 2017; Goula et al., 2016). In addition, the variation in tannin content between varieties, irrespective of the drying method could be due to the number of seeds per berry rather than the amount of tannin per seed, considering that 750 g/ kg DM of extractable berry proanthocyanidins are found in the seeds at harvest.

The decline in total phenolic content between freeze- and oven-dried Sauvignon Blanc and Shiraz in the current study was moderate (279 – 396 g/ kg DM) compared to the 87 – 95% range decline as reported by Goula et al. (2016). The temperature regimes (60 and 85 °C) used in the two studies could have been the reason for such differences as it is known that both magnitude and duration of heating has a strong influence on phenolic stability (Larrauri et al., 1997; Tseng and Zhao, 2012). Another explanation could be that freeze-dried tissue has greater porosity (i.e., 80 –

90%) compared to other drying techniques, therefore, accelerating solvent diffusion and subsequently increases the transfer of phenolic compounds to the solvent (Çoklar and Akbulut, 2017). Preceding freeze drying is the freezing of the samples at -20 °C, temperatures which form ice crystals and eventually degradation of cell membrane, allowing for greater release of phenolics from cell vacuoles (Tomaz et al., 2017).

The proanthocyanidin content is comparable to previous studies (6.9 – 139 g/ kg DM) (Hixson et al., 2016) but lower than other reports (216 – 516 g/ kg DM) (Rondeau et al., 2013). Proanthocyanidin content decline between freeze-dried Sauvignon Blanc and other methods was lower (147 – 371 g/ kg DM) compared to total phenolics. This agrees with Larrauri et al. (1997), despite them employing higher temperatures (100 and 140 °C). One reason could be the relatively heat-stable nature of proanthocyanidins because of the high degree of polymerization.

The lower anthocyanin content for Sauvignon Blanc variety regardless of drying method was expected because the red color (i.e., anthocyanin) is characteristic of red varieties (Downey et al., 2006). Overall, the anthocyanin content compared to other phenolic groups could possibly be attributed to the degradative nature of anthocyanins through oxidative mechanisms involving polyphenol oxidase (Lingua et al., 2016). The fact that sun and oven drying resulted in significant reductions in phenolic content could be exploited as a way of reducing their negative effects when GP is fed to ruminants. Proanthocyanidin content above 60 g/ kg DM in feed has been associated with reduced intake, nutrient digestibility and animal performance, despite tolerance of 80 g/ kg DM depending on the forage species (Patra and Saxena, 2009) and animal factors (i.e., species and breed (Mlambo and Mapiye, 2015).

Overall, interactive effects between grape variety and drying method were observed for both DPPH and FRAP. Çoklar and Akbulut (2017) and Larrauri et al. (1997) came to the same conclusion regarding the thermal influence on phenolics of grape and its byproducts and its subsequent reduction in antioxidant activities. The higher antioxidant capacity displayed by freeze-dried varieties for DPPH and FRAP is related to the high phenolic content observed in these freeze-dried varieties, especially the high proanthocyanidin content for freeze-dried Sauvignon Blanc. Proanthocyanidin is a type of flavonoid known for its high antioxidant properties related to the high degree of hydroxyl groups (Negro et al., 2003). The observation that phenolics and FRAP values were closely clustered could imply a causal-effect relationship between them. For example, the observed relationship between total flavonoids and FRAP from the PCA results, further supports the association between them. The observation that phenolics and DPPH clusters were slightly distant can be explained by the different mechanism of action between the assays, especially the steric hindrance of bulky ring adducts and multiple rings (e.g., proanthocyanidin) towards the DPPH radical compared to the simple phenols and ascorbic acid (Schaich et al., 2015). It is known that the antioxidant activity of grape-derived products is influenced, not only by the total phenolic content but also the composition, both of which are mainly influenced by grape variety. It has been suggested that the bioactive constituents of white GP extract are more heat resistant than those from red ones, possibly due to their reflective nature in white grapes (Larrauri et al., 1997).

There is a general consensus among researchers that temperature has a negative influence on the antioxidant activity of biological material (Çoklar and Akbulut, 2017; Larrauri et al., 1998; Tseng and Zhao, 2012). High temperatures are associated with poor quality products due to

Maillardation and enzymatic reactions, which also contribute to the reduced antioxidant properties of phenolic compounds (Larrauri et al., 1998). On the contrary, Nicoli et al. (1997) reported that oxidation of some phenolic compounds might temporarily result in the generation of new antioxidant metabolites. This could be a plausible explanation observed for high radical scavenging activity percentage for both sun-dried treatments of Shiraz and Sauvignon Blanc. Based on the structure-activity relationships, phenolic compounds antioxidant capacity is increased with the high degree of hydroxylation; a function of the numbers and positions of the hydroxyl groups within the carboxyl functional group (Tseng and Zhao, 2012). Therefore, the inclusion of GP in ruminant diets has potential to reduce oxidative stress and improve the shelf stability of meat due to the antioxidative nature of phenolics as reported by (Zhao et al., 2018) in grape pomace-fed lambs.

Grape pomace variety was significant towards microbial species abundance and diversity (i.e., evenness and richness). The observed decline in species abundance in GP treated feed could be because of the toxic nature of phenolic compounds like tannins (García-Lomillo and González-SanJosé, 2017). This could be related to their protein-binding capacity resulting in complex with proteins in the cell wall and membrane of bacteria causing morphological changes of the cell wall and the extracellular enzymes secreted (Patra and Saxena, 2009). The increased diversity indices (evenness and richness) for GP supplemented feeds compared to ruminal fluid could be linked to the contribution of nutrients from the lamb feed. This is also revealed by the PCA, which showed closeness of the treatments on species abundance and diversity indices. De Nardi et al. (2016) reported that polyphenol treatment increased the richness and diversity of rumen microbiota of beef cattle. However, Jones et al. (1994) reported contrasting results of Saifoin proanthocyanidins

on individual bacterial species where there was an increase in *Butryrivibrio fibrisolvens* A38 and *Streptococcus bovis* 45S1 but little changes were observed for *Prevotella ruminicola* B14 or *Ruminobacter amylophilus* WP225. Likewise, Vasta et al. (2010) also observed an increase in *B. fibrosolvens* and a decline in *B. proteoclasticus* populations after feeding quebracho tannins to sheep. The mechanism is based on phenolic compound toxicity, particularly, proanthocyanidins, on bacterial species involved in FA biohydrogenation. This occurs through selective inhibition of cell wall synthesis (morphological changes), metal chelation, impeding transport of nutrients into the cell or retarding the growth of the microbes (Vasta et al., 2010). Additionally, it might be related to the development of tolerance and adaptive mechanisms by bacteria, for instance, the secretion of exo-polysaccharide forming a protective layer around the cells, modification/ degradation of tannins, dissociation of tannin-substrate complexes, tannin inactivation by high-affinity binders, and modification of cell membrane and metal ion sequestration by these bacteria (Patra and Saxena, 2009).

The high similarity (i.e., low R- ANOSIM) between the two red varieties was expected as they are genetically related (Jackson and Lombard, 1993). The microbial diversity evaluation revealed that the abundance of ruminal species is reduced by addition of GP, with a clear distinction between the microbial populations in white and red varieties. It is important to further research on the antimicrobial effects of sun-dried GP to evaluate changes in specific microbial population through advanced methods such as high-throughput amplicon sequencing (Jami et al., 2014), which enables the identification of individual species, especially those involved in biohydrogenation. This is because ARISA only gives bacterial diversity and community fingerprinting but does not allow for the identification of the individual species (Jami et al., 2014).

Incorporation of GP in ruminant diets is likely to boost the proportions of health-beneficial PUFA and their biohydrogenation intermediate products in meat (Gravador et al., 2015). The resultant high degree of FA unsaturation may increase their susceptibility to oxidation resulting in production of off-flavors, loss of health benefits and reduced shelf-life (Gravador et al., 2015). These negative effects may, however, be counteracted by the natural polyphenolic antioxidants and antibacterial present in GP (García-Lomillo and González-SanJosé, 2017; Tseng and Zhao, 2012). Although varietal differences reduced microbial abundance and improved diversity, there was no distinct variety which performed better across treatments. Regarding dehydration methods, despite freeze- and oven-drying having better bioactive profiles and biological activities, they have relatively higher operating costs and energy consumption compared to sun drying (Thi and Hwang, 2016). Solar energy is a promising technique of extending the shelf-life of fresh GP, especially in areas where plentiful sunshine is available all year round mostly because of its lower cost and efficient retention of bioactive profile and biological activities (Ng et al., 2018). In that regard, feeding sun-dried GP as a natural dietary source of antioxidants and antimicrobials to ruminants is, therefore, likely to simultaneously enhance meat healthfulness and shelf-life and reduce the negative environmental effects associated with the dumping of fresh GP.

4.5 Conclusions

Based on the present findings, grape variety and drying had profound influence on the bioactive profile and biological activities. Relative to other drying × variety interactions, freeze-dried Shiraz had the best fatty acid profile and the highest contents of phenolic compounds. Freeze-dried Sauvignon Blanc had the highest proanthocyanidins and exhibited the best antioxidant activity. Overall, sun- and oven-drying methods showed similar efficiency in retaining bioactive

profile and biological activities. To improve the adoption and utilization of GP in the livestock feed industry, further studies to determine the digestibility, growth performance, meat quality and shelf-stability of meat from ruminants fed sun-dried GP are warranted.

4.6 References

Al Juhaimi, F., Geçgel, Gülcü, M., Hamurcu, M., Özcan, M.M., 2017. Bioactive properties, fatty acid composition and mineral contents of grape seed and oils. South African J. Enol. Vitic. 38, 103–108.

AOAC (Association of Official Analytical Chemists), 2002. Official methods of analysis, 17th ed. Association of Official Analytical Chemists Inc., Arlington, Virginia, USA.

Benzie, I.F.F., Strain, J.J., 1996. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": The FRAP assay. Anal. Biochem. 239, 70–76.

Beres, C., Costa, G.N.S., Cabezudo, I., da Silva-James, N.K., Teles, A.S.C., Cruz, A.P.G., Mellinger-Silva, C., Tonon, R. V., Cabral, L.M.C., Freitas, S.P., 2017. Towards integral utilization of grape pomace from winemaking process: A review. Waste Manag. 68, 581-594.

Cardinale, M., Brusetti, L., Quatrini, P., Borin, S., Puglia, A.M., Rizzi, A., Zanardini, E., Sorlini, C., Corselli, C., Daffonchio, D., 2004. Comparison of different primer sets for use in automated ribosomal intergenic spacer analysis of complex bacterial communities. Appl. Environ. Microbiol. 70, 6147–6156.

Choe, E., Oh, S., 2013. Effects of water activity on the lipid oxidation and antioxidants of dried laver (*Porphyra*) during storage in the dark. J. Food Sci. 78, C1144–C1151.

Çoklar, H., Akbulut, M., 2017. Effect of sun, oven and freeze-drying on anthocyanins, phenolic compounds and antioxidant activity of Black grape (Ekşikara) (*Vitis vinifera* L.). South African J. Enol. Vitic. 38, 264–272.

CSIRO (Commonwealth Scientific and Industrial Research Organization), 2007. Nutrient Requirements of Domesticated Ruminants. CSIRO Publishing, Collingwood VIC 3066, Australia.

Culman, S.W., Bukowski, R., Gauch, H.G., Cadillo-Quiroz, H., Buckley, D.H., 2009. T-REX: Software for the processing and analysis of T-RFLP data. BMC Bioinformatics 10, 171.

De Nardi, R., Marchesini, G., Li, S., Khafipour, E., Plaizier, K.J.C., Gianesella, M., Ricci, R., Andrighetto, I., Segato, S., 2016. Metagenomic analysis of rumen microbial population in dairy heifers fed a high grain diet supplemented with dicarboxylic acids or polyphenols. BMC Vet. Res. 12, 29.

Downey O., M., Dokoozlian K., N., Krstic P., M., 2006. Cultural practice and environmental impacts on the flavonoid composition of grapes and wine: A review of recent research. Am. J. Enol. Vitic. 57, 257–268.

Gan, H., Charters, E., Driscoll, R., Srzednicki, G., 2017. Effects of drying and blanching on the retention of bioactive compounds in ginger and turmeric. Horticulturae 3, 13.

García-Lomillo, J., González-SanJosé, M.L., 2017. Applications of wine pomace in the food industry: Approaches and functions. Compr. Rev. Food Sci. Food Saf. 16, 3–22.

Giusti, M.M., Wrolstad, R.E., 2001. Characterization and measurement of anthocyanins by UV–visible spectroscopy, in: Current Protocols in Food Analytical Chemistry. Wiley-Blackwell, New York, USA, p. F1: F1.2.

Goering, H.K., Van Soest, P.J., 1970. Forage Fiber Analyses (Apparatus, Reagents, Procedures and some Applications) - Agricultural handbook no.379. US Dept. of Agriculture, Washington DC., USA.

Goula, A.M., Thymiatis, K., Kaderides, K., 2016. Valorization of grape pomace: Drying behavior and ultrasound extraction of phenolics. Food Bioprod. Process. 100, 132–144.

Gravador, R.S., Luciano, G., Jongberg, S., Bognanno, M., Scerra, M., Andersen, M.L., Lund, M.N., Priolo, A., 2015. Fatty acids and oxidative stability of meat from lambs fed carob-containing diets. Food Chem. 182, 27–34.

Hall, M.B., 2009. Determination of starch, including maltooligosaccharides, in animal feeds: Comparison of methods and a method recommended for AOAC collaborative study. J. AOAC Int. 92, 42–49.

Hammer, Ø., Harper, D.A.T., Ryan, P.D., 2001. PAST: Paleontological Statistics Software Package for education and data analysis. Palaeontol. Electron. 4(1), 1–9.

Hixson, J.L., Jacobs, J.L., Wilkes, E.N., Smith, P.A., 2016. Survey of the variation in grape marc condensed tannin composition and concentration and analysis of key compositional factors. J. Agric. Food Chem. 64, 7076–7086.

Jackson, D.I., Lombard, P.B., 1993. Environmental and management practices affecting grape composition and wine quality - A review. Am. J. Enol. Vitic. 44, 409–430.

Jami, E., Shterzer, N., Mizrahi, I., 2014. Evaluation of Automated Ribosomal Intergenic Spacer Analysis for bacterial fingerprinting of rumen microbiome compared to pyrosequencing technology. Pathogens 3, 109–120.

Jones, G.A., McAllister, T.A., Muir, A.D., Cheng, K.J., 1994. Effects of sainfoin (*Onobrychis viciifolia* Scop.) condensed tannins on growth and proteolysis by four strains of ruminal bacteria. Appl. Environ. Microbiol. 60, 1374-1378.

Karioti, A., Hadjipavlou-Litina, D., Mensah, M.L.K., Fleischer, T.C., Skaltsa, H., 2004. Composition and antioxidant activity of the essential oils of *Xylopia aethiopica* (Dun) A. rich. (*Annonaceae*) leaves, stem bark, root bark, and fresh and dried fruits, growing in Ghana. J. Agric. Food Chem. 52, 8094–8098.

Larrauri, J.A., Rupérez, P., Saura-Calixto, F., 1997. Effect of drying temperature on the stability of polyphenols and antioxidant activity of red grape pomace peels. J. Agric. Food Chem. 45, 1390–1393.

Larrauri, J.A., Sánchez-Moreno, C., Saura-Calixto, F., 1998. Effect of temperature on the free radical scavenging capacity of extracts from red and white grape pomace Peels. J. Agric. Food Chem. 46, 2694–2697.

Lingua, M.S., Fabani, M.P., Wunderlin, D.A., Baroni, M. V., 2016. From grape to wine: Changes in phenolic composition and its influence on antioxidant activity. Food Chem. 208, 228–238.

Lutterodt, H., Slavin, M., Whent, M., Turner, E., Yu, L., 2011. Fatty acid composition, oxidative stability, antioxidant and antiproliferative properties of selected cold-pressed grape seed oils and flours. Food Chem. 128, 391–399.

Makkar, H.P.S., Siddhuraju, P., Becker, K., 2007. Tannins, in: Makkar, H.P.S., Siddhuraju, P., Becker, K. (Eds.), Plant Secondary Metabolites. Humana Pres Inc., New Jersey, USA, pp. 67–82.

Mapiye, C., Aldai, N., Turner, T.D., Aalhus, J.L., Rolland, D.C., Kramer, J.K.G., Dugan, M.E.R.,

2012. The labile lipid fraction of meat: From perceived disease and waste to health and opportunity. Meat Sci. 92, 210–220.

Mapiye, C., Vahmani, P., Mlambo, V., Muchenje, V., Dzama, K., Hoffman, L.C., Dugan, M.E.R., 2015. The trans-octadecenoic fatty acid profile of beef: Implications for global food and nutrition security. Food Res. Int. 76, 992–1000.

Meï, C., Michaud, M., Cussac, M., Albrieux, C., Gros, V., Maréchal, E., Block, M.A., Jouhet, J., Rébeillé, F., 2015. Levels of polyunsaturated fatty acids correlate with growth rate in plant cell cultures. Sci. Rep. 5, 15207.

Mertens, D.R., 2002. Gravimetric determination of amylase-treated neutral detergent fiber in feeds with refluxing in beakers or crucibles: Collaborative study. J. AOAC Int. 85, 1217–1240.

Mlambo, V., Mapiye, C., 2015. Towards household food and nutrition security in semi-arid areas: What role for condensed tannin-rich ruminant feedstuffs? Food Res. Int. 76, 953–961.

Negro, C., Tommasi, L., Miceli, A., 2003. Phenolic compounds and antioxidant activity from red grape marc extracts. Bioresour. Technol. 87, 41–44.

Ng, M.X., Tham, T.C., Gan, S.H., Chua, L.S., Aziz, R., Baba, M.R., Chuah Abdullah, L., Chin, N.L., Ong, S.P., Law, C.L., 2018. *Clinacanthus nutans* Lindau: Effects of drying methods on the bioactive compounds, color characteristics, and water activity. Dry. Technol. 36, 146–159.

Nicoli, M.C., Anese, M., Parpinel, M.T., Franceschi, S., Lerici, C.R., 1997. Loss and/or formation of antioxidants during food processing and storage. Cancer Lett. 114, 71–74.

Patra, A.K., Saxena, J., 2009. Dietary phytochemicals as rumen modifiers: A review of the effects on microbial populations. Antonie Van Leeuwenhoek. 96, 363–375.

Porter, L.J., Hrstich, L.N., Chan, B.G., 1986. The conversion of procyanidins and prodelphinidins to cyanidin and delphinidin. Phytochemistry 25, 223–230.

Raffrenato, E., Van Amburgh, M.E., 2011. Technical note: Improved methodology for analyses of acid detergent fiber and acid detergent lignin. J. Dairy Sci. 94, 3613–3617.

Rondeau, P., Gambier, F., Jolibert, F., Brosse, N., 2013. Compositions and chemical variability of grape pomaces from French vineyard. Ind. Crops Prod. 43, 251–254.

Sabir, A., Unver, A., Kara, Z., 2012. The fatty acid and tocopherol constituents of the seed oil extracted from 21 grape varieties (*Vitis* spp.). J. Sci. Food Agric. 92, 1982–1987.

Schaich, K.M., Tian, X., Xie, J., 2015. Reprint of "Hurdles and pitfalls in measuring antioxidant efficacy: A critical evaluation of ABTS, DPPH, and ORAC assays." J. Funct. Foods 18, 782–796.

Setati, M.E., Jacobson, D., Andong, U.C., Bauer, F., 2012. The vineyard yeast microbiome, a mixed model microbial map. PLoS One 7, e52609.

Shokryazdan, P., Rajion, M.A., Meng, G.Y., Boo, L.J., Ebrahimi, M., Royan, M., Sahebi, M., Azizi, P., Abiri, R., Jahromi, M.F., 2017. Conjugated linoleic acid: A potent fatty acid linked to animal and human health. Crit. Rev. Food Sci. Nutr. 57, 2737–2748.

Thi, N. Do, Hwang, E.S., 2016. Effects of drying methods on contents of bioactive compounds and antioxidant activities of black chokeberries (*Aronia melanocarpa*). Food Sci. Biotechnol. 25, 55–61.

This, P., Lacombe, T., Thomas, M.R., 2006. Historical origins and genetic diversity of wine grapes. Trends Genet. 22, 511-519.

Tomaz, I., Šeparović, M., Štambuk, P., Preiner, D., Maletić, E., Karoglan Kontić, J., 2017. Effect of freezing and different thawing methods on the content of polyphenolic compounds of red grape skins. J. Food Process. Preserv. 42, e13550.

Tseng, A., Zhao, Y., 2012. Effect of different drying methods and storage time on the retention of bioactive compounds and antibacterial activity of wine grape pomace (Pinot Noir and Merlot). J. Food Sci. 77, H192–H201.

Vasta, V., Yáñez-Ruiz, D.R., Mele, M., Serra, A., Luciano, G., Lanza, M., Biondi, L., Priolo, A., 2010. Bacterial and protozoal communities and fatty acid profile in the rumen of sheep fed a diet containing added tannins. Appl. Environ. Microbiol. 76, 2549–2555.

Wannamethee, S.G., Jefferis, B.J., Lennon, L., Papacosta, O., Whincup, P.H., Hingorani, A.D., 2018. Serum conjugated linoleic acid and risk of incident heart failure in older men: The British regional heart study. J. Am. Heart Assoc. 7, e006653.

Wen, X., Zhu, M., Hu, R., Zhao, J., Chen, Z., Li, J., Ni, Y., 2016. Characterization of seed oils from different grape cultivars grown in China. J. Food Sci. Technol. 53, 3129–3136.

Yang, J., Martinson, T.E., Liu, R.H., 2009. Phytochemical profiles and antioxidant activities of wine grapes. Food Chem. 116, 332–339.

Yi, C., Shi, J., Kramer, J., Xue, S., Jiang, Y., Zhang, M., Ma, Y., Pohorly, J., 2009. Fatty acid composition and phenolic antioxidants of winemaking pomace powder. Food Chem. 114, 570–576.

Zhang, N., Hoadley, A., Patel, J., Lim, S., Li, C., 2017. Sustainable options for the utilization of solid residues from wine production. Waste Manag. 60, 173–183.

Zhao, J.X., Li, Q., Zhang, R.X., Liu, W.Z., Ren, Y.S., Zhang, C.X., Zhang, J.X., 2018. Effect of dietary grape pomace on growth performance, meat quality and antioxidant activity in ram lambs. Anim. Feed Sci. Technol. 236, 76–85.

Chapter 5 Effect of grape (*Vitis vinifera* L.) pomace supplementation on nutrient utilization in finisher lambs[4]

ABSTRACT

The objective of the study was to evaluate the effects of feeding varying levels of sun-dried red grape pomace (GP; *Vitis vinifera* L. cultivar Pinotage) on nutrient digestibility, rumen fermentation, microbial nitrogen (N) supply, N retention and utilization efficiency in lambs. Twenty-one Dohne merino wether lambs (6.0 ± 1.0 mo and 51.6 ± 4.70 kg initial body weight) were randomly assigned to three diets containing 0, 100 and 200 g GP per kg of diet dry matter in a total mixed ration. The experiment consisted of 14 days for adaptation to the diets and 7 days for data and sample collection. Intake of neutral detergent fiber (aNDFom) and starch decreased linearly ($P \leq 0.05$) while ether extract intake increased linearly ($P \leq 0.05$) with the addition of GP. Apparent total tract aNDFom digestibility decreased linearly ($P \leq 0.05$) with increasing levels of GP. Total volatile fatty acids (VFA) production was quadratically ($P \leq 0.05$) influenced by GP

[4] A version of this chapter is under review:
Effect of grape (*Vitis vinifera* L.) pomace supplementation on nutrient utilization in finisher lambs. Small Ruminant Research

addition. Increasing levels of GP led to a linear decrease ($P \leq 0.05$) in concentrations of butyrate and valerate. Allantoin, microbial N supply, total purine derivatives excreted and absorbed linearly declined ($P \leq 0.05$) with GP addition. Nitrogen retention and the efficiency of N utilization were not influenced by diet ($P > 0.05$). Overall, addition of GP in the lamb finisher diet reduced carbohydrate intake, total VFA concentration, microbial N yield, total purine derivatives excreted but it did not adversely affect N retention and utilization efficiency.

Keywords

Efficiency of nitrogen utilization, Grape pomace, Nutrient digestibility, Proanthocyanidins, Purine derivatives.

5.1 Introduction

About one third of the 2310 million tons of global cereal grain harvest are used for livestock feed (FAO, 2013). Although ruminants compete with humans for cereals, their uniqueness emanates from their ability to utilize fibrous feedstuffs owing to the consortium of rumen micro-organisms (Van Soest, 1994). Variability in nutrient composition and nutritive value, high lignin content and, at times, the presence of plant secondary metabolites, especially when fed at high levels may, however, limit fiber utilization by rumen microbes (Makkar, 2003; McSweeney et al., 2001). The same factors may also affect digestibility and availability of other nutrients, especially nitrogen (N) through the inadequate supply of ammonia-N (NH_3-N) in the rumen leading to suboptimal growth of microbes (Chandrasekharaiah et al., 2011). Subsequently, the low microbial growth may reduce absorption and tissue deposition of nutrients because of the binding effects of

secondary metabolites, especially proanthocyanidins to digesta proteins, reduced activity of digestive enzymes and/ or impairment of the functional activity of the intestine (Makkar, 2003; Waghorn, 2008). In addition to digestion of fiber, rumen microbes also provide more than 60% of amino acids to the host animal (Hackmann and Firkins, 2015). Sustainable ruminant livestock production systems should, therefore, promote efficient utilization of fibrous and nitrogenous resources for food production and minimize the impact of these resources on the environment.

Grapes are one of the most economically valued horticultural fruits globally. More than two-thirds of global grape production of 77.3 metric tons (OIV, 2015) are destined for the wine industry, generating substantial quantities of byproducts, including pomace, which subsequently pose serious economic, environmental and social challenges (Beres et al., 2017; Zhang et al., 2017). In the Mediterranean, grape pomace (GP) has traditionally been incorporated in growing lamb diets as a fibrous source up to 300 g/ kg of dietary dry matter (DM) during periods of scarce feed supply (Manso et al., 2016; Zepf and Jin, 2013). Inclusion of GP in ruminant diets has, however, generally been limited by high contents of lignin and proanthocyanidins (Alipour and Rouzbehan, 2007; Baumgärtel et al., 2007). Overall, high content of neutral detergent fiber (NDF; > 300 g/ kg DM), particularly lignin (Arelovich et al., 2008; Harper and McNeill, 2015; Smith, 2008) and proanthocyanidins (> 60 g/ kg) (Abarghuei et al., 2010; Baumgärtel et al., 2007) in lamb finisher diets may reduce nutrient intake and digestibility. Increased nutrient intake and digestibility have, however, been reported with low to moderate concentrations of NDF (150 – 300 g/ kg DM) (Arelovich et al., 2008; Harper and McNeill, 2015; Smith, 2008) and proanthocyanidins (20 – 50g/ kg DM; Baumgärtel et al., 2007; Mueller-Harvey, 2006) in the diet. The high contents of proanthocyanidins in feeds can efficiently and cost-effectively be reduced by sun-drying (Ben

Salem et al., 1999) or co-feeding (Waghorn, 2008) GP with other ingredients that have little or no phenolic compounds.

Utilization of winery byproducts in ruminant diets in wine producing developing countries is scant. In South Africa, for example, there is little if any information about the use of pomace from the local bred red grape variety, *Vitis vinifera* L. cultivar Pinotage, in ruminant diets. In addition, there is limited data on the nutrient digestibility, ruminal fermentation and the efficiency of N utilization of GP-based lamb finisher diets. Overall, phytochemicals including proanthocyanidins have been used as natural feed additives with the aim of manipulating rumen fermentation, especially to increase efficiency of N utilization and animal performance (Abarghuei et al., 2010; Ishida et al., 2015; Rajabi et al., 2017). The diverse nature of bioactive compounds in GP could be effective in modulating ruminal fermentation and nutrient utilization, subsequently improving animal production (Guerra-Rivas et al., 2017; Manso et al., 2016). Based on the nutrient composition, the low proanthocyanidin content, drying costs, its uniqueness as a locally bred variety and abundance (Chapter 3 and 4), sun-dried Pinotage was selected for subsequent trials. The objective of the present study was, therefore, to assess *in vivo* nutrient digestibility, ruminal fermentation parameters, microbial protein synthesis and N balance in Dohne Merino wether lambs fed finisher diets containing different levels of red grape (*Vitis vinifera* L. cultivar Pinotage) pomace.

5.2 Materials and methods

5.2.1 Study site

The present study was conducted in September of 2017 at Welgevallen Experimental Farm (33.9427° S, 18.8664° E; Stellenbosch University, Stellenbosch, South Africa). All procedures involving the use and care of animals were approved by the Stellenbosch University Animal Ethics Committee (SU-ACUD16-00143).

5.2.2 Experimental diets, animals and experimental design

Fresh red GP (*Vitis vinifera* L. cv. Pinotage) was sourced at Bellevue Wine Estate (33.879866° S, 18.763768° E; Stellenbosch, South Africa). The pomace was immediately sun-dried on 6 mm polyethylene canvas sheets for 7 days to ensure a moisture content of less than 10% (Chapter 3). The dried pomace was milled (Drotsky M16 Hammer mill, Aktief (Pty) Ltd, Johannesburg, South Africa) to pass through a 4-mm sieve and used as an ingredient in lamb finisher diets. Three diets containing 0 (control), 100 and 200 g GP/kg diet DM (Table 5.1). The pellet diameter of 5 mm and average length of 30 mm length, respectively. The diets were made isoenergetic and isonitrogenous by substitution of fibrous ingredients with GP (National Research Council, 2007).

Twenty-one Dohne Merino wether lambs (6.0 ± 1.0 mo and 51.6 ± 4.70 kg initial body weight) were confined to individual pens (2 m^2) with slated floors. Prior to the digestibility trial, lambs were fed a high grain diet similar to the control diet for 60 days. Thereafter, the lambs were adapted to the experimental diets for 14 days prior to a 7-day total collection period. Prior to this, the animals were treated for external (Inverject, FarmVet, South Africa) and internal (ByBoost Lamb

and Kid + copper, Bayer (Pty) Ltd., South Africa) parasites and vaccinated against enterotoxaemia and Pasteurella (Enteroprotect P 100, DP, South Africa). The dietary treatments were assigned to lambs (7 lambs/ treatment) in a completely randomized design. The lambs were fed once daily at 0800 h and received the diets *ad libitum* as total mixed rations. Diets were provided at 110% of the previous daily intake. Clean, fresh water was available at all times.

Table 5.1 Feed ingredients and chemical composition of experimental diets

Item	Inclusion of grape pomace (g/ kg)			
	0	100	200	
Ingredients				
Sun-dried Pinotage meal[1]	0	100	200	
Lucerne meal	200	200	200	
Soybean hulls	37.6	48.2	5.6	
Hominy chop (maize bran)	50	50	0	
Defatted maize germ	150	150	130	
Wheat bran middlings	90.9	46.8	0	
Oat bran	50	0	0	
Maize meal	281	292	325	
Megalac[2]	0	0	20.7	
Molasses syrup	40	40	40	
Fish meal[3]	0	0	3.81	
Lupins	13.3	5.97	0	
Soybean oil cake[4]	38.7	33.3	46.9	
Limestone fine	24.8	18.8	13.3	
Salt fine	8.2	4.4	4.2	
Toxin binder (Mycosorb A)	1	1	1	
Mold inhibitor (Technigard)	0.5	0.5	0.5	
Sal CURB® S liquid mix[5]	5	0	0	
Vitamin/ Mineral Premix[6]	7	7	7	
Dust binder (Dustex)	2	2	2	
Chemical composition				SEM[12]
DM (g/ kg as-fed basis)	880	878	881	0.55
Ash	89.7	71.8	75.6	1.05
Organic matter	910	928	924	1.05
Crude protein	173	179	174	1.95
Ether extract	37.1	49.8	63.4	0.90
Starch	285	283	284	2.12
aNDFom[7]	300	289	243	6.27
ADFom[8]	190	166	182	3.77
Lignin (sa.) [9]	24.3	47.0	60.3	2.10
Metabolizable Energy (MJ/ kg DM)[10]	10.9	11.6	11.4	0.08
Non-fiber carbohydrates[11]	401	410	444	6.43
Total tannins (g gallic acid equivalent/ kg DM)	0	34.7	59.4	2.06
Proanthocyanidins (g cyanidin chloride equivalent/ kg DM)	0	12.3	25.7	0.24

[1] Chemical composition GP meal: 919 g/ kg DM; ash, 56.8 g/ kg; crude protein, 107 g/ kg DM; ether extract, 89.3 g/ kg DM; starch, 69.2 g/ kg DM, aNDFom, 401 g/ kg; total tannins, 143 g/ kg DM; proanthocyanidins, 64.9 g/ kg DM.
[2] Megalac: A high-energy rumen-protected fat supplement (calcium salt of palm fatty acids)
[3] Fish meal containing 650 g/ kg protein.
[4] Soybean oil cake: Soybean containing 470 g/ kg protein.
[5] Sal CURB® S liquid mix: antimicrobial used to control *Salmonella* contamination.
[6] Vitamin/ mineral premix (i.e., MW Sheep PX with Monesin 68813). The composition of the vitamin/ premix not included because of an agreement by the feed manufacturer not to disclose confidential information.
[7] aNDFom: neutral detergent fiber assayed with heat stable amylase and expressed exclusive of ash.
[8] ADFom: Acid detergent fiber expressed exclusive of ash.
[9] Lignin (sa): Lignin determined by solubilization of cellulose with sulfuric acid.
[10] Estimated according to CSIRO (2007).
[11] Non fiber carbohydrates: Calculated as: 1000– (aNDFom g/ kg + crude protein g/ kg + ether extract g/ kg + ash g/ kg).
[12] SEM: Standard error of means. Number of samples analyzed per treatment = 4.

5.2.3 In vivo nutrient digestibility, ruminal fermentation, microbial nitrogen supply and nitrogen balance

Three days prior to the first collection period, each lamb was fitted with a strap-on canvas fecal collection bag. Urine was collected through a urinary tube using a funnel-shaped latex bag attached to the dorsal side of the animal. During the collection period (7 days), feed offered, refusals, fecal and urine samples were collected before feeding. Representative samples of feed and refusals were composited pending chemical analyses. To estimate voluntary intake for each lamb, the nutrient content in refusals was subtracted from that offered in feed. Total fecal matter voided by each lamb was weighed and 10% of daily amount was stored at -20 °C. Feed, refusals and fecal samples were dried in a forced air-oven at 60 °C for 72 h, ground using a Wiley mill (Model 4, Thomas Scientific, Swedesboro, NJ, USA) to pass a 1-mm sieve.

Total urine for each lamb was collected in 4 L buckets containing 100 mL of 2 M sulfuric acid to maintain an acidic pH (< 3) to prevent bacterial destruction of purine derivatives and the volatilization of NH_3. To prevent the precipitation of purine derivatives in urine during storage, 50 mL of the daily amount was sampled and diluted fivefold with distilled water (Rajabi et al., 2017). Twenty mL of the undiluted urine was kept at -20 °C for determination of total nitrogen.

The animals were transported to an abattoir 70 km away from the Experimental Farm. The animals were kept in lairage for 16 h before slaughter. Thirty minutes after slaughter, rumen contents were collected from each lamb according to Chaves et al. (2011) and pH was measured using a portable pH meter (Crison PH25 pH meter, Lasec, South Africa). The rumen contents were mixed thoroughly and then filtered through four layers of cheesecloth into 50 mL Greiner centrifuge tubes (Merck KGaA, Darmstadt, Germany). The tubes were capped and placed on ice

and immediately transferred to the laboratory. Ruminal fluid was centrifuged at 1000 × g for 10 min (4 °C). One mL of the supernatant was preserved with 0.1 mL of 5% phosphoric acid (v/ v) for NH_3-N analyses. For volatile fatty acids (VFA) concentration determination, 0.1 mL of 25% (w/ v) metaphosphoric acid was added to 0.9 mL of supernatant. Both samples were stored at -20 °C pending analyses.

5.2.4 Chemical analyses and calculations

Dry matter (DM; method 934.01), ash (method; 942.05) and ether extract (EE; method 920.39) contents in feed, refusals and fecal samples were determined according to the AOAC (2002) procedures. Nitrogen content for feed, feces and urine was analyzed using the Dumas method with a macro-Nitrogen analyzer (LECO® FP528, LECO Corporation, Miami, USA). Nitrogen content was used for crude protein (CP) determination by multiplying with a factor of 6.25. Starch was measured using a commercial assay (Total Starch Megazyme kit KTSTA, Megazyme International Ireland Ltd., Wicklow, Ireland) following the method for samples containing glucose and/ or maltodextrins (Hall, 2009). Neutral detergent fiber (aNDFom) was determined using heat-stable alpha-amylase and addition of sodium sulfite (Mertens et al., 2002). Acid detergent fiber (ADFom) was performed according to AOAC (method 962.09; 2002). Lignin (sa.) was analyzed according to Goering and Van Soest (1970) as modified by Raffrenato and Van Amburgh (2011). Nutrient digestibility was calculated as: [nutrient intake (g/ day) – nutrient in fecal matter (g/ day)]/ nutrient intake (g/ day). The true digestibility of organic matter (OM) was estimated assuming that only the aNDFom fraction of feces originated from feed (Orlandi et al., 2015; Van Soest, 1994) as: [OM intake (g/ day) − fecal NDF (g/ day)]/ OM intake (g/ day). Total tannin content was measured by

the Folin-Ciocalteu colorimetric method according to Makkar et al. (2007) and expressed as g gallic acid equivalent per kg DM. Proanthocyanidin content was measured using the procedure of Porter et al. (1986) and results expressed as g cyanidin chloride equivalent per kg DM.

Ammonia-N was determined by colorimetry (Broderick and Kang, 1980). Volatile fatty acids were quantified using a gas chromatography (GC; Thermo Scientific™ TRACE™ 1300, Switzerland) fitted with Thermo TriPlus RSH Autosampler. A Phenomenex Zebron ZB-FFAP capillary GC column (30 m length × 0.25 mm internal diameter × 0.25 µm film thickness) was used. Crotonic acid was used as the internal standard. The injection volume was 1 µL and the run time was 18 min. Thermo Scientific Xcalibur™ Software was used to calculate VFA concentrations. The VFA were expressed as mmol/ L, with the individual VFA later converted to mmol/ 100 mmol of the total VFA.

Urinary purine derivatives (PD) were determined by the spectrophotometric method (Chen and Gomes, 1992). The total excretion of PD was calculated as the sum of allantoin, uric acid, xanthine and hypoxanthine amounts excreted in urine. The quantitative relationship between absorption of microbial purines and excretion of PD in urine was determined using the nonlinear equation:

$Y = 0.84\ X + (0.150\ W^{0.75}\ e^{-0.25X})$, where Y is the excretion of microbial purine derivative in urine, X is the absorption of microbial purine derivative, both in mmol/ d and $W^{0.75}$ is the metabolic body weight (kg) of the animal. The Newton-Raphson iteration process was performed to calculate the absorbed microbial purines based on the above equation by means of the following relationship:

$X_{(n+1)} = X_n - \left[\frac{f(X_n)}{f'(X_n)}\right]$, where $f(X) = 0.84\ X + 0.150\ W^{0.75}\ e^{-0.25X} - Y$ and the derivative of $f(X)$: $f'(X) = (0.84 - 0.038\ W^{0.75}\ e^{-0.25X})$. Finally, the intestinal flow of microbial N produced was estimated as:

$$\text{Microbial N (g N/ d)} = \frac{X\ (\text{mmol/d}) \times 70}{0.116 \times 0.83 \times 1000} = 0.727\ X.$$

Nitrogen retention was calculated as the difference between daily N intake and daily N excretion (i.e., urinary plus fecal N).

5.2.5 Statistical analyses

All data were analyzed using response surface regression (RSREG; SAS, 2012) to determine the relationship between the response variables and the inclusion level of GP in lamb finisher diets using the following model:

$Y = \beta_0 + \beta_1 X + \beta_2 X^2 + \varepsilon$, where Y is the observation (nutrient digestibility, ruminal fermentation parameter, microbial N supply and N retention), β_0, β_1, and β_2 are the regression coefficients and X is the inclusion level of sun-dried GP meal and ε is the random error. Treatment means were generated using least-square means procedure of SAS (2012). Probability (P) values ≤ 0.05 were considered significant and a tendency was reported if $0.05 < P \leq 0.10$.

5.3 Results

5.3.1 Chemical composition of experimental diets

The energy and protein contents were similar across diets (Table 5.1). However, ether extract, lignin, non-fiber carbohydrates, total tannins and proanthocyanidins increased with addition of GP

to the diet (Table 5.1). Neutral detergent fiber declined with increasing levels of GP in the diet (Table 5.1).

5.3.2 Effect of feeding red grape pomace on nutrient intake and digestibility in lambs

Table 5.2 shows the results for the intake and digestibility of nutrients. Dry matter, OM, and CP intakes were not affected by the inclusion of GP in lamb diets ($P > 0.05$). The addition of GP in the diet resulted in a linear decrease ($P \leq 0.05$) in aNDFom and starch intake. There was a positive linear ($P \leq 0.05$) relationship between EE intake and inclusion level of GP. Regarding apparent total tract digestibility, only aNDFom decreased linearly ($P \leq 0.05$) with increasing levels of GP.

5.3.3 Effect of feeding red grape pomace on ruminal fermentation parameters in lambs

The effects of increasing inclusion levels of GP in the diet on ruminal fermentation parameters is shown in Table 5.3. Grape pomace inclusion level had no effect ($P > 0.05$) on ruminal pH. Ammonia-N exhibited a tendency for a linear decrease ($P \leq 0.10$) as the level of GP increased in the diet. The concentration of total VFA was quadratically ($P \leq 0.05$) influenced by addition of GP in the diet with a maximum concentration of 67.1 mmol/ L obtained at an optimum inclusion level of 151 g/ kg DM. Increasing levels of GP in the diet led to linear increases ($P \leq 0.05$) in molar proportions of butyrate and valerate.

5.3.4 Effect of feeding red grape pomace on purine derivatives and nitrogen balance in lambs

The daily excretion of allantoin declined linearly ($P \leq 0.05$) with increasing level of GP (Table 5.4). The rest of the purine derivatives (i.e., uric acid and xanthine plus hypoxanthine) were not influenced by GP addition ($P > 0.05$; Table 3.4). The total purine derivative excreted, and microbial N supply showed a negative linear ($P \leq 0.05$) response to the increasing level of GP in the diet. No dietary effects were observed for fecal and urinary N excretion, N retention and the efficiency of N utilization ($P > 0.05$). Overall, animals on all dietary treatments were in a positive N balance, with the N voided through feces higher than urinary N.

Table 5.2 Effect of red grape pomace on nutrient intake and digestibility in lambs

Item	Grape pomace inclusion			SEM[c]	P value		
	0	100	200		Diet	Linear[d]	Quadratic[d]
Nutrient intake (g/ kg DM)							
Dry matter	1192	1144	1127	35.2	0.424	0.213	0.720
Organic matter	1085	1062	1042	32.3	0.654	0.364	0.964
Crude protein	204	201	197	6.10	0.703	0.412	0.904
aNDFom	357	331	274	10.1	<0.0001	<0.01	0.228
Starch	319	272	264	8.92	0.001	0.001	0.098
Ether extract	44.2	57.0	71.5	1.65	<0.0001	<0.01	0.676
Apparent total tract digestibility[a] (g/ kg DM)							
Dry matter	628	626	632	29.4	0.988	0.927	0.900
Organic matter	774	753	741	21.0	0.543	0.282	0.859
Crude protein	722	739	694	17.7	0.228	0.291	0.172
aNDFom	719	729	632	26.1	0.031	0.029	0.110
Starch	963	957	961	4.91	0.704	0.855	0.420
Ether extract	925	924	929	7.42	0.866	0.723	0.693
True organic matter digestibility[b]	908	916	903	7.93	0.542	0.692	0.307

[a] Apparent digestibility = [nutrient intake (g/ d) – nutrient in feces (g/ d)]/ nutrient intake (g/ d).
[b] True OM digestibility = [OM intake (g/ d) − fecal NDF (g/ d)]/ OM intake (g/ d).
[c] SEM, standards error of means.
[d] Probability of treatment effect by regression analysis.

Table 5.3 Effect of red grape pomace on ruminal fermentation parameters in lambs

Item	Grape pomace inclusion (g/ kg DM)			SEM [a]	P value		
	0	100	200		Diet	Linear [b]	Quadratic [b]
pH	6.81	6.78	6.76	0.06	0.226	0.120	0.461
Ammonia-N (mg/ L)	75.3	66.8	51.5	5.64	0.189	0.072	0.957
Total VFA (mmol/ L)	55.3	65.7	65.8	2.45	0.001	0.001	0.044
VFA (mmol/ 100 mmol)							
Acetate	50.9	50.4	48.2	1.57	0.452	0.239	0.678
Propionate	24.2	24.5	22.3	0.97	0.266	0.203	0.308
Butyrate	14.4	15.3	17.4	0.90	0.082	0.031	0.605
Isobutyrate	3.87	3.53	4.25	0.36	0.378	0.455	0.241
Valerate	3.18	3.19	4.06	0.21	0.010	0.007	0.105
Isovalerate	3.46	3.07	3.72	0.27	0.256	0.500	0.134
Acetate: Propionate	2.11	2.12	2.16	0.12	0.964	0.803	0.928

[a] SEM, standards error of means.
[b] Probability of treatment effect by regression analysis.

Table 5.4 Effect of red grape pomace on purine derivatives, microbial nitrogen supply and nitrogen retention in lambs

Item	Grape pomace inclusion (g/ kg DM)			SEM[c]	P value		
	0	100	200		Diet	Linear[d]	Quadratic[d]
Urinary excretion, mmol/ d							
Allantoin	11.7	9.77	9.58	0.55	0.031	0.015	0.261
Uric acid	2.27	2.42	2.34	0.24	0.974	0.896	0.853
Xanthine + hypoxanthine	1.12	1.36	1.37	0.20	0.627	0.385	0.690
Total purine derivatives excreted	15.1	13.6	13.3	0.54	0.061	0.029	0.349
Total purine derivative absorbed	17.9	16.1	15.8	0.65	0.061	0.029	0.353
Microbial N supply, g N/ d DOMI[a]	13.0	11.7	11.5	0.47	0.061	0.029	0.353
Nitrogen balance (g/ d)							
Fecal N	9.48	8.83	9.60	0.66	0.678	0.902	0.389
Urinary N	14.5	14.2	14.5	0.41	0.868	0.990	0.600
N retention[b]	7.96	8.61	7.25	0.99	0.632	0.619	0.418
Efficiency of N utilization by animal	24.6	26.9	22.9	0.03	0.556	0.654	0.329

[a] Digestible organic matter intake (g/ kg DM) = [OM intake (g) − fecal OM (g)]/ DM intake (g).
[b] Nitrogen retention = daily nitrogen intake – daily nitrogen excretion (fecal + urine).
[c] SEM, standards error of means.
[d] Probability of treatment effect by regression analysis.

5.4 Discussion

Overall, substitutions in the diets were mainly for wheat bran middlings, oat bran and soybean hulls. A rumen-protected fat supplement (Megalac) was added as an energy source in the 200 g/ kg GP diet to make the diets isoenergetic while fishmeal was added in the same diet to balance the nitrogen across diets. Diet planning simulated what happens at commercial level where several ingredients are included in the diet based on their cost and/ or efficacy. The lack of significant differences among the treatments regarding the intake of DM, OM and CP could be an indication that GP inclusion had no adverse effects on palatability and consumption by lambs. Similar findings on DM and OM intake were reported in literature (Ferreira, 2004; Ishida et al., 2015) with the supplementation of GP in sheep diets up to 500 g GP / kg of diet DM (Ferreira, 2004). The observed linear decline in intake of aNDFom and starch with addition of GP could be attributed to a combination of the increasing levels of lignin, proanthocyanidins and possibly crude fat in the diet, mostly contributed by the elevated GP content and the addition of Megalac. The binding effect of proanthocyanidins with biomolecules is known to have greater preference to CP than fiber (Le Bourvellec and Renard, 2012). In the current study, a contrast to this phenomenon was observed. There is no immediate explanation to this observation but could be linked to the interactions between lignin and proanthocyanidins with polysaccharides, subsequently acting as physical barriers to microbial enzymes reaching their target polysaccharides (Jung and Allen, 1995; Moore and Jung, 2001). The majority of research has focused on protein-proanthocyanidin complexes and little data is available in the literature regarding the interaction between polysaccharides with polyphenols (Le Bourvellec and Renard, 2012). The higher the lignin content, the less NDF intake by the animal as the digesta is retained longer within the rumen

(Mertens, 1994; Van Soest, 1994). Furthermore, the presence of proanthocyanidins might have had an additive effect on decreased polysaccharide intake and digestibility. Proanthocyanidins produce astringent sensations and bitter taste, which, at higher levels, induce aversion and also interact with salivary glycoproteins, modulating their post-ingestive effects (Frutos et al., 2004; Makkar, 2003).

The reduced intake of the aNDFom, starch and ether extract with GP inclusion level may partly be related to the increase of fat from the pomace. Grape seed accounts for nearly half of the pomace, of which between 600 – 800 g/ kg DM are polyunsaturated fatty acids, notably linoleic acid (García-Lomillo and González-SanJosé, 2017). That could partly justify the increase in fat with the addition of GP to the diet. High fat content may indirectly suppress appetite, however, the mechanisms involved have not been clearly identified in ruminants (Harvatine and Allen, 2006). Detrimental effects usually occur with fat levels >50 g/ kg DM, with polyunsaturated fatty acids exerting the most negative effect on ruminal microbes, thereby reducing digestion efficiency (Allen, 2000; Palmquist and Jenkins, 2017). Alternatively, fats may form a coat around fiber particles creating a barrier between feed and rumen microbes, eventually suppressing ruminal digestion (Palmquist and Jenkins, 2017). Although the current study did not measure dietary fatty acid profile, recent research is showing that nutrient digestibility is likely dependent upon fatty acid composition and its interaction with dietary components, more important than the total fat (de Souza et al., 2018; Piantoni et al., 2015). This likely occurs through the effect of different fatty acids on release of the gut peptide and the subsequent effect on retention time of digesta in the rumen (Piantoni et al., 2015).

The observed decrease in apparent total tract aNDFom digestibility could have also been jointly influenced by lignin, proanthocyanidins and fat as discussed for nutrient intake (Frutos et al., 2004). Furthermore, Pantoja et al. (1994) reported that aNDFom digestibility could be reduced presumably due to the degree of high degree of unsaturation of the added fat. Proanthocyanidins tend to have a greater negative effect on protein digestibility compared to other nutrients including polysaccharides (i.e., aNDFom and starch) because of the presence of multiple binding sites for macromolecules (Jayanegara and Palupi, 2010). The mechanisms of polyphenol-polysaccharide associations would be the same as for proteins through non-covalent bonds (hydrogen bonding, hydrophobic bonding, van der Waals forces) or irreversible covalent bonds (Le Bourvellec and Renard, 2012). The negative effect of proanthocyanidins on nutrient digestibility is more pronounced when the content is > 60 g/ kg DM (Mueller-Harvey, 2006; Waghorn, 2008).

Although, diet had no effect on ruminal pH, it was observed that the values were within the normal range (5.5 – 6.8) (McDonald et al., 2011; Van Soest, 1994). Maintenance of pH within the normal range is important for efficient functioning of the rumen microbial ecosystem. The reported linear tendency of NH_3-N concentration to decrease could be attributed to the effects of GP polyphenols and their effects on protein and fiber digestion as discussed earlier. These findings concur with research by Foiklang et al. (2016) and Ishida et al. (2015) who supplemented GP to diets of swamp buffaloes and lambs, respectively. The NH_3-N concentration reported for the current study are slightly below the optimum level of 85 to 300 mg/ L for microbial growth (McDonald et al., 2011). However, McDonald et al. (2011) states that a minimum level of 50 mg/ L of NH_3-N is still sufficient for microbial growth, albeit their slow growth. It is suspected that the

concentrations were low not because of dietary treatments but by the long duration in which the rumen was sampled (16 h after slaughter) as opposed to collecting the sample through a fistula.

The observed quadratic response of total VFA for the GP treatments suggest a higher microbial activity compared to the control, especially with regards to the tannin-resistant gram-negative bacteria (Smith et al., 2005; Smith and Mackie, 2004). However, as the proanthocyanidins content increases in the diet, there is a reduction in fiber digestion which may subsequently reduce the energy available to rumen microbes for protein synthesis (Makkar, 2003) thereby reducing microbial protein supply to the host animal. The total VFA were slightly below the normal range of 70 – 150 mmol/ L (McDonald et al., 2011), which could have been affected by the fact the rumen contents were collected from lambs after 16 h-period off feed before slaughter. The high fat content contributed by the pomace in the diet could have also contributed to the higher total VFA. In a meta-analysis, Patra (2013) reported that increasing fat in the diet contributes to the overall total VFA concentration.

The increase in butyrate content across treatments may have arisen from its interconversions with acetate, through the various intermediate substrates, to allow for the anabolism of anaerobic bacteria (Hackmann and Firkins, 2015). Although, the effect of acetate was not significant, the marginal decline for the 200 g/ kg GP could have led to the higher content of butyrate for the same diet. Besides these interconversions, high butyrate-producing bacteria, such as the *Butyrivibrio fibrisolvens* increase in the rumen of animals fed tannin-containing feeds (Buccioni et al., 2012; Ohkawara et al., 2005) could have also influenced the high production. The effects of GP on valerate proportion among the dietary treatments are of low magnitude from a practical point of

view. It is usually produced from the deamination of amino acids (Van Soest, 1994) and contributes only a small fraction of the total VFA.

The linear decline of allantoin by 16.4 to 18.1% relative to the control could be due to the increase in the lignin and proanthocyanidins content of GP-based diets. A similar pattern was observed in the total excreted and absorbed purine and microbial N supply. As explained earlier, the interaction of biomolecules with lignin and proanthocyanidins, particularly protein could have reduced protein degradation in the rumen with most dietary amino acids flowing to the duodenum (Makkar, 2003; McSweeney et al., 2001). A positive relationship between urinary purine derivative excretion and allantoin in particular, the principal purine metabolite, can be used as a good indicator of microbial purine catabolism, subsequently microbial protein synthesis in ruminants (Chen and Gomes, 1992). There is a presumption that the proanthocyanidin-protein bond dissociates in the acidic environment of the abomasum, but the covalent bond of this complex is thought to be irreversibly bound (McMahon et al., 2000) and may not liberate the bound amino acid.

The supplementation of GP in lamb finisher diets did not influence the nitrogen balance parameters (fecal N and urinary N). Similar findings were reported by Ishida et al. (2015) upon feeding dietary GP at 90 g/ kg. These findings are in contrast to the observations by Abarghuei et al. (2010) who reported that retained N was decreased in sheep fed grape pomace because of the lower microbial protein synthesis in the rumen and the increased fecal N losses. The efficiency of N utilization falls within the range (10 – 40%) for ruminants (Calsamiglia et al., 2010). Given that GP addition had neutral effect on the efficiency of N utilization, it can be considered as cheap alternative feed source in lamb feeding programs.

5.5 Conclusions

Overall, addition of GP in lamb finisher diets reduced carbohydrate intake, total VFA concentration, microbial N supply, total purine derivatives excreted and absorbed, but did not adversely affect N retention and utilization efficiency. A follow up study to determine how the observed decreases in nutrient intake and digestibility, VFA concentration and microbial N yield when feeding lambs varying levels of GP would influence growth performance, carcass and meat quality is important.

5.6 References

Abarghuei, M.J., Rouzbehan, Y., Alipour, D., 2010. The influence of the grape pomace on the ruminal parameters of sheep. Livest. Sci. 132, 73–79.

Alipour, D., Rouzbehan, Y., 2007. Effects of ensiling grape pomace and addition of polyethylene glycol on in vitro gas production and microbial biomass yield. Anim. Feed Sci. Technol. 137, 138–149.

Allen, M.S., 2000. Effects of diet on short-term regulation of feed intake by lactating dairy cattle. J. Dairy Sci. 83, 1598–1624.

AOAC (Association of Official Analytical Chemists), 2002. Official Methods of Analysis, 17th ed. Association of Official Analytical Chemists Inc., Arlington, Virginia, USA.

Arelovich, H.M., Abney, C.S., Vizcarra, J.A., Galyean, M.L., 2008. Effects of dietary neutral detergent fiber on intakes of dry matter and net energy by dairy and beef cattle : Analysis of published data. Prof. Anim. Sci. 24, 375–383.

Baumgärtel, T., Kluth, H., Epperlein, K., Rodehutscord, M., 2007. A note on digestibility and energy value for sheep of different grape pomace. Small Rumin. Res. 67, 302–306.

Ben Salem, H., Nefzaoui, A., Ben Salem, L., Tisserand, J.L., 1999. Intake, digestibility, urinary excretion of purine derivatives and growth by sheep given fresh, air-dried or polyethylene glycol-treated foliage of *Acacia cyanophylla* Lindl. Anim. Feed Sci. Technol. 78, 297–311.

Beres, C., Costa, G.N.S., Cabezudo, I., da Silva-James, N.K., Teles, A.S.C., Cruz, A.P.G., Mellinger-Silva, C., Tonon, R. V., Cabral, L.M.C., Freitas, S.P., 2017. Towards integral utilization of grape pomace from winemaking process: A review. Waste Manag. 68, 581–594.

Broderick, G.A., Kang, J.H., 1980. Automated simultaneous determination of ammonia and total amino acids in ruminal fluid and in vitro media. J. Dairy Sci. 63, 64–75.

Buccioni, A., Decandia, M., Minieri, S., Molle, G., Cabiddu, A., 2012. Lipid metabolism in the rumen: New insights on lipolysis and biohydrogenation with an emphasis on the role of endogenous plant factors. Anim. Feed Sci. Technol. 174, 1–25.

Calsamiglia, S., Ferret, A., Reynolds, C.K., Kristensen, N.B., Van Vuuren, A.M., 2010. Strategies for optimizing nitrogen use by ruminants. Animal 4, 1184–1196.

Chandrasekharaiah, M., Thulasi, A., Suresh, K.P., Sampath, K.T., 2011. Rumen degradable nitrogen requirements for optimum microbial protein synthesis and nutrient utilization in sheep fed on finger millet straw (*Eleucine coracana*) based diet. Anim. Feed Sci. Technol. 163, 130–135.

Chaves, A.V., Dugan, M.E.R., Stanford, K., Gibson, L.L., Bystrom, J.M., McAllister, T.A., Van Herk, F., Benchaar, C., 2011. A dose-response of cinnamaldehyde supplementation on intake, ruminal fermentation, blood metabolites, growth performance, and carcass characteristics of growing lambs. Livest. Sci. 141, 213–220.

Chen, X.B., Gomes, M.J., 1992. Estimation of microbial protein supply to sheep and cattle based on urinary excretion of purine derivatives- An overview of the technical details. Rowett Research Institute, Bucksburn, Aberdeen, UK.

CSIRO (Commonwealth Scientific and Industrial Research Organization), 2007. Nutrient Requirements of Domesticated Ruminants. CSIRO Publishing, Collingwood VIC 3066, Australia.

de Souza, J., Preseault, C.L., Lock, A.L., 2018. Altering the ratio of dietary palmitic, stearic, and oleic acids in diets with or without whole cottonseed affects nutrient digestibility, energy partitioning, and production responses of dairy cows. J. Dairy Sci. 101, 172–185.

FAO (Food and Agriculture Organization), 2013. Food Outlook: Biannual Report on Global Food Markets.

Ferreira, A.V., 2004. Nutritive value of red vine husks and pips for sheep. S. Afr. J. Anim. Sci. 34, 23–25.

Foiklang, S., Wanapat, M., Norrapoke, T., 2016. Effect of grape pomace powder, mangosteen peel powder and monensin on nutrient digestibility, rumen fermentation, nitrogen balance and microbial protein synthesis in dairy steers. Asian-Australasian J. Anim. Sci. 29, 1416–1423.

Frutos, P., Hervás, G., Mantecón, A.R., 2004. Tannins and ruminant nutrition. Spanish J. Agric. Res. 2, 191–202.

García-Lomillo, J., González-SanJosé, M.L., 2017. Applications of wine pomace in the food industry: Approaches and functions. Compr. Rev. Food Sci. Food Saf. 16, 3–22.

Goering, H.K., Van Soest, P.J., 1970. Forage fiber analyses (Apparatus, reagants, procedures and some applications)- Agricultural handbook no.379. US Dept. of Agriculture, Washington

DC., USA.

Guerra-Rivas, C., Gallardo, B., Mantecón, Á.R., del Álamo-Sanza, M., Manso, T., 2017. Evaluation of grape pomace from red wine byproduct as feed for sheep. J. Sci. Food Agric. 97, 1885–1893.

Hackmann, T.J., Firkins, J.L., 2015. Maximizing efficiency of rumen microbial protein production. Front. Microbiol. 6, 465.

Hall, M.B., 2009. Determination of starch, including maltooligosaccharides, in animal feeds: Comparison of methods and a method recommended for AOAC collaborative study. J. AOAC Int. 92, 42–49.

Harper, K., McNeill, D., 2015. The role iNDF in the regulation of feed intake and the importance of its assessment in subtropical ruminant systems (the role of iNDF in the regulation of forage intake). Agriculture 5, 778–790.

Harvatine, K.J., Allen, M.S., 2006. Effects of fatty acid supplements on feed intake, and feeding and chewing behavior of lactating dairy cows. J. Dairy Sci. 89, 1104–1112.

Ishida, K., Kishi, Y., Oishi, K., Hirooka, H., Kumagai, H., 2015. Effects of feeding polyphenol-rich winery wastes on digestibility, nitrogen utilization, ruminal fermentation, antioxidant status and oxidative stress in wethers. Anim. Sci. J. 86, 260–269.

Jayanegara, A., Palupi, E., 2010. Condensed tannin effects on nitrogen digestion in ruminants: A meta-analysis from in vitro and in vivo studies. Media Peternak. 33, 176–181.

Jung, H.G., Allen, M.S., 1995. Characteristics of plant cell walls affecting intake and digestibility of forages by ruminants. J. Anim. Sci. 73, 2774–2790.

Le Bourvellec, C., Renard, C.M., 2012. Interactions between polyphenols and macromolecules

quantification methods and mechanisms. Crit. Rev. Food Sci. Nutr. 52, 213–248.

Makkar, H.P.S., 2003. Effects and fate of tannins in ruminant animals, adaptation to tannins, and strategies to overcome detrimental effects of feeding tannin-rich feeds. Small Rumin. Res. 49, 241–256.

Makkar, H.P.S., Siddhuraju, P., Becker, K., 2007. Tannins, in: Makkar, H.P.S., Siddhuraju, P., Becker, K. (Eds.), Plant Secondary Metabolites. Humana Pres Inc., New Jersey, USA, pp. 67–82.

Manso, T., Gallardo, B., Guerra-Rivas, C., 2016. Modifying milk and meat fat quality through feed changes. Small Rumin. Res. 142, 31–37.

McDonald, P., Edwards, R.A., Greenhalgh, J.F.D., Morgan, C.A., Sinclair, L.A., Wilkinson, R.G., 2011. Animal Nutrition, 7th ed. Pearson Education Limited, Harlow, United Kingdom.

McMahon, L.R., McAllister, T.A., Berg, B.P., Majak, W., Acharya, S.N., Popp, J.D., Coulman, B.E., Wang, Y., Cheng, K., 2000. A review of the effects of forage condensed tannins on ruminal fermentation and bloat in grazing cattle. Can. J. Plant Sci. 80, 469–485.

McSweeney, C.S., Palmer, B., McNeill, D.M., Krause, D.O., 2001. Microbial interactions with tannins: Nutritional consequences for ruminants. Anim. Feed Sci. Technol. 91, 83–93.

Mertens, D.R., 1994. Regulation of forage Intake, in: Forage Quality, Evaluation, and Utilization. American Society of Agronomy, Crop Science Society of America, Soil Science Society of America, Madison, WI, pp. 494–532.

Mertens, D.R., Allen, M., Carmany, J., Clegg, J., Davidowicz, A., Drouches, M., Frank, K., Gambin, D., Garkie, M., Gildemeister, B., Jeffress, D., Jeon, C.S., Jones, D., Kaplan, D., Kim, G.N., Kobata, S., Main, D., Moua, X., Paul, B., Robertson, J., Taysom, D., Thiex, N.,

Williams, J., Wolf, M., 2002. Gravimetric determination of amylase-treated neutral detergent fiber in feeds with refluxing in beakers or crucibles: Collaborative study. J. AOAC Int. 85, 1217–1240.

Moore, K.J., Jung, H.-J.G., 2001. Lignin and fiber digestion. J. Range Manag. 54, 420–430.

Mueller-Harvey, I., 2006. Unravelling the conundrum of tannins in animal nutrition and health. J. Sci. Food Agric. 86, 2010–2037.

National Research Council, 2007. Nutrient Requirements of Small Ruminants. National Academy Press, Washington DC, USA.

Ohkawara, S., Furuya, H., Nagashima, K., Asanuma, N., Hino, T., 2005. Oral administration of Butyrivibrio fibrisolvens, a butyrate-producing bacterium, decreases the formation of aberrant crypt foci in the colon and rectum of mice. J. Nutr. 135, 2878–2883.

OIV (Organisation Internationale de la Vigne et du Vin), 2015. World Vitiviniculture Situation (2015), OIV Statistical Report on World Vitiviniculture.

Orlandi, T., Kozloski, G.V., Alves, T.P., Mesquita, F.R., Ávila, S.C., 2015. Digestibility, ruminal fermentation and duodenal flux of amino acids in steers fed grass forage plus concentrate containing increasing levels of Acacia mearnsii tannin extract. Anim. Feed Sci. Technol. 210, 37–45.

Palmquist, D.L., Jenkins, T.C., 2017. A 100-Year Review: Fat feeding of dairy cows. J. Dairy Sci. 100, 10061–10077.

Pantoja, J., Firkins, J.L., Eastridge, M.L., Hull, B.L., 1994. Effects of fat saturation and source of fiber on site of nutrient Digestion and milk production by lactating dairy cows. J. Dairy Sci. 77, 2341–2356.

Patra, A.K., 2013. The effect of dietary fats on methane emissions, and its other effects on digestibility, rumen fermentation and lactation performance in cattle: A meta-analysis. Livest. Sci. 155, 244–254.

Piantoni, P., Lock, A.L., Allen, M.S., 2015. Saturated fat supplementation interacts with dietary forage neutral detergent fiber content during the immediate postpartum and carryover periods in Holstein cows: Production responses and digestibility of nutrients. J. Dairy Sci. 98, 3309–3322.

Porter, L.J., Hrstich, L.N., Chan, B.G., 1986. The conversion of procyanidins and prodelphinidins to cyanidin and delphinidin. Phytochemistry 25, 223–230.

Raffrenato, E., Van Amburgh, M.E., 2011. Technical note: Improved methodology for analyses of acid detergent fiber and acid detergent lignin. J. Dairy Sci. 94, 3613–3617.

Rajabi, M., Rouzbehan, Y., Rezaei, J., 2017. A strategy to improve nitrogen utilization, reduce environmental impact, and increase performance and antioxidant capacity of fattening lambs using pomegranate peel extract. J. Anim. Sci. 95, 499–510.

SAS, 2012. SAS/SAST User's Guide. Statistics, Version 9.4. Cary, NC: USA.

Smith, A.H., Mackie, R.I., 2004. Effect of condensed tannins on bacterial diversity and metabolic activity in the rat gastrointestinal tract. Appl. Environ. Microbiol. 70, 1104–1115.

Smith, A.H., Zoetendal, E., Mackie, R.I., 2005. Bacterial mechanisms to overcome inhibitory effects of dietary tannins. Microb. Ecol. 50, 197–205.

Smith, P.S., 2008. Dietary fibre requirements of feedlot lambs. University of the Free State.

Van Soest, P.J., 1994. Nutritional Ecology of the Ruminant, 2nd ed. Cornell University Press, Ithaca, USA.

Waghorn, G., 2008. Beneficial and detrimental effects of dietary condensed tannins for sustainable sheep and goat production-Progress and challenges. Anim. Feed Sci. Technol. 147, 116–139.

Zepf, F., Jin, B., 2013. Bioconversion of grape marc into protein rich animal feed by microbial fungi. Chem. Eng. Process Tech. 1, 1011.

Zhang, N., Hoadley, A., Patel, J., Lim, S., Li, C., 2017. Sustainable options for the utilization of solid residues from wine production. Waste Manag. 60, 173–183.

Chapter 6 Grape pomace (*Vitis vinifera* L. cv. Pinotage) supplementation in lamb diets: Effects on growth performance, carcass and meat quality[5]

ABSTRACT

This study investigated the effects of feeding graded levels of sun-dried red grape pomace (GP; 0, 50, 100, 150 and 200 g GP / kg of diet DM) on growth, carcass and meat physicochemical quality attributes of Dohne Merino lambs for 42 days. Dry matter intake increased quadratically with a critical value (i.e., optimum inclusion level) of 113 g GP / kg of diet ($P \leq 0.05$). Diet exhibited similar quadratic responses for average daily gain, and live, hot and cold carcass weights with optimum inclusion levels at 96, 97, 122 and 121 g GP / kg of diet, respectively ($P \leq 0.05$). Overall, meat quality traits were not negatively affected by GP inclusion ($P > 0.05$). Gross profit was influenced by diet, with an optimum inclusion level at of 122 g GP / kg of diet (quadratic; $P \leq$

[5] A version of this chapter has been published:

Chikwanha, O.C., Muchenje, V., Nolte, J.E., Dugan, M.E.R., Mapiye, C., 2019. Grape pomace (Vitis vinifera L. cv. Pinotage) supplementation in lamb diets: Effects on growth performance, carcass and meat quality. Meat Sci. 147, 6–12. doi:10.1016/j.meatsci.2018.08.017

0.05). Overall, inclusion of between 100 and 120 g GP / kg of diet in lamb finishing diets at the expense of oat bran and wheat bran middling's improved lamb productivity, without compromising meat quality.

Keywords

Carcass traits, Grape pomace, Gross profit, Growth performance, Lamb, Meat quality.

6.1 Introduction

Grape pomace (GP), a byproduct of the wine industry, has for a long time been undervalued and treated as a waste due to lack of alternative uses with economic benefits (García-Lomillo and González-SanJosé, 2017). Pomace contributes the bulk of the solid fraction, approximately 25% of the pressed grapes, and mainly comprises skin (~510 g/ kg DM), seeds (~470 g/ kg DM) and stalks (~ 20 g/ kg DM) (Beres et al., 2017; Zhang et al., 2017). Although, GP is not hazardous, if not managed properly it has negative environmental effects including phytotoxic effects to crops, surface and ground water pollution, depletion of oxygen in soil and ground waters by tannins, and attraction of flies and pests that may spread diseases (Beres et al., 2017; Dwyer et al., Zhang et al., 2017). Depletion of oxygen occurs through the activity of phenol oxidase enzymes, which are responsible for degradation of phenolics (Min et al., 2015). The increased attention to sustainable agricultural practices demands finding alternative uses for this byproduct.

It is estimated that only 3% of the global GP is used as animal feed (Brenes et al., 2016), with Australia, a major wine producing country using 13% as fodder (Zhang et al., 2017). The low usage of GP in livestock production is due to its high content of lignin and polymeric polyphenols,

mainly proanthocyanidins which are associated with reduced digestibility through the inhibition of cellulolytic and proteolytic enzymes (Baumgärtel et al., 2007).

In pursuit of sustainable and economically viable ruminant livestock systems, many farmers worldwide are under increasing pressure to maximize the use of available agricultural byproduct-based diets for their livestock (Nkosi and Meeske, 2010) without compromising production performance and meat quality traits. Grape pomace has potential to be incorporated in ruminant diets because of its moderate to high protein content (90 – 250 g/ kg DM) (García-Lomillo and González-SanJosé, 2017). Grape pomace is a waste product with low cost, giving it an added advantage over hay, which has to be purchased and often at a relatively higher cost (Moate et al., 2014). Zepf and Jin (2013) recomended that GP inclusion in ruminant diets should be, however, limited to not more than 30% of the diet because of its high lignin and phenolic contents.

Despite the high phenolic content in GP, sun-drying (Pirmohammadi et al., 2007) or co-feeding (Waghorn, 2008) in combination with other feeds that have little or no phenolic compounds effectively reduces the levels of these compounds and improve feed intake, nitrogen and fiber digestibility (Kumar and D'Mello, 1995). Moderate levels of phenolic compounds, particularly tannins are recognized for their nutrient-binding properties, which improve nitrogen utilization by reducing ruminal protein degradation and, thus, increasing by-pass protein supply and subsequently growth (Perez-Maldonado and Norton, 1996). Furthermore, the bioactive compounds in GP could improve meat yield because of the increased feed intake and quality through antioxidative and antimicrobial properties of phenolics (Guerra-Rivas et al., 2017; Manso et al., 2016). Inclusion of GP in sheep diets also has the potential to reduce the cost of diets (Manso et al., 2016). Grape pomace produced in South Africa, especially, from the local varieties have

been relatively unexploited by the local meat industry as a source of animal feed, mostly owing to scant information on the benefits of GP as an ingredient in feedlot diets. Thus, the objective of the current study was to evaluate the effects of inclusion level of sun-dried red grape (*Vitis vinifera* L. cv. Pinotage) on growth, carcass and meat quality traits of feedlot-reared Dohne Merino lambs.

6.2 Materials and methods

The experiment was conducted between August and September 2017 at Welgevallen Experimental Farm (33.9427° S, 18.8664° E; Stellenbosch University, South Africa). The study protocol for care and use of animals used in the experiment was approved by the Stellenbosch University Animal Ethics Committee (SU-ACUD16-00143).

6.2.1 Preparation of diets

Fresh red GP (*Vitis vinifera* L. cv. Pinotage) was provided by Bellevue Wine Estate (33.879866° S, 18.763768° E) in Stellenbosch. Immediately after pressing, the GP was spread onto 6 mm polyethylene canvas sheets under sunlight. The pomace was turned daily for 7 days to ensure moisture content was below 10% and milled to pass through a 4 mm sieve. Five pelleted (5 mm, at 80 °C) total-mixed diets were prepared by a registered commercial feed manufacturer: control (no GP) and four treatments with 50, 100, 150 and 200 g GP / kg of diet DM, respectively (Table 6.1). All diets were formulated to be isoenergetic and isonitrogenous (National Research Council, 2007).

6.2.2 Diets, experimental design and animal management

Forty castrated Dohne Merino lambs averaging 32 ± 1.7 kg and 3 - 4 months old were purchased from a commercial feedlot in Hermon, South Africa. All the animals were treated for external (Inverject, FarmVet, South Africa) and internal (ByBoost Lamb and Kid + copper, Bayer (Pty) Ltd., South Africa) parasites, and vaccinated against enterotoxaemia and Pasteurella (Enteroprotect P 100, DP, South Africa). Lambs were housed individually in slated floor pens (2 m^2). Lambs were assigned to dietary treatments (8 lambs/ treatment) in a completely randomized design. Data collection commenced after a 14-day adaptation period and lasted for 42 days during which growth performance was monitored. Feed and clean fresh water were offered every morning ad libitum. Feed offered, and corresponding orts were recorded daily to estimate voluntary DM intake (DMI) and representative samples collected weekly and stored at -20 °C for further analysis. Lambs were fasted for 16 h at the beginning of the trial and also before slaughter to determine the full-body weights of the animals. Weekly weights were taken before morning feeding to determine average daily gain (ADG) and feed efficiency (gain to feed ratio).

6.2.3 Chemical analyses of feed and orts

Dry matter (method 934.01), ash (method 942.05) and ether extract (EE; method 920.39) contents were determined according to the AOAC (2002) procedures. Total nitrogen content was analyzed using the Dumas method with a macro-Nitrogen analyzer (LECO® FP528, LECO Corporation, Miami, USA). Crude protein (CP) was calculated by multiplying the nitrogen content by a factor of 6.25. Starch was measured using a commercial assay (Total Starch Megazyme kit KTSTA, Megazyme International Ireland Ltd., Wicklow, Ireland), following the method for

samples containing glucose and/ or maltodextrins (Hall, 2009). Neutral detergent fiber (aNDFom) was determined using heat-stable alpha-amylase and addition of sodium sulfite (Mertens, 2002). Lignin (sa.) was analyzed according to Goering and Van Soest (1970) as modified by Raffrenato and Van Amburgh (2011). Neutral detergent fiber and lignin (sa.) were expressed exclusive of ash. Total tannin content was measured by the Folin–Ciocalteu colorimetric method (Makkar, 2007). Gallic acid standard curve (0.02 – 0.10 mg/ mL) was used and total tannins were expressed as gram gallic acid equivalent per 100 g DM. Proanthocyanidin content was measured using the procedures of Porter et al. (1986) and results expressed as gram cyanidin chloride equivalent per 100 g DM. All results were means of four replicates.

6.2.4 Slaughter and carcass measurements

At the end of the feeding trial, the lambs were transported to a commercial abattoir 70 km from the experimental site. Lambs were electrically stunned (200 V applied for 4 seconds) and slaughtered by exsanguination after 16 h off feed, with access to water only. After dressing, the carcasses were immediately weighed to obtain hot carcass weights. The temperature and pH (Crison PH25 pH meter, Lasec, South Africa) were measured in the *longissimus thoracis et lumborum* (LTL) muscle between the 12^{th} and 13^{th} ribs, 45 min and 24 h post-slaughter. The carcasses were classed based on the South African Meat Industry Company (2006) by assessing age, fatness and conformation. Carcass fatness was classed on a scale of 0 – 6 (0 = no visual fat cover, 1 = very lean, 2 = lean, 3 = medium, 4 = fat, 5 = over-fat, and 6 = excessively over-fat). Conformation was assessed based on the following classes: 1 (very flat), 2 (flat), 3 (medium), 4 (round) and 5 (very round). The carcasses were chilled at ± 3°C for 24 h and weighed again to

determine cold carcass weight. Dressing percentage was calculated as hot carcass weight divided by live weight. The LTL was removed 24 h post mortem for meat quality analyses.

6.2.5 Meat sampling and physicochemical characteristics

After removal of the LTL from the carcass, six slices (2 cm thick) were cut and randomly assigned for drip loss and color, cooking loss and Warner-Bratzler Shear force (WBSF) determination and proximate analyses. Physicochemical analyses were performed in duplicate, except for WBSF.

6.2.5.1 Color and drip loss

Color measurements were performed directly on the meat surface after blooming for 30 min. Lightness (L*), redness (a*) and yellowness (b*) parameters were recorded using the CIELAB color meter: Color-guide 45°/ 0° colorimeter (BYK-Gardner GmbH, Gerestried, Germany) with a 11-mm diameter aperture, using an illuminant/ observer of D65/ 10° observer settings. The measurements were taken three times at different locations per sample. The hue angle (H°) and chroma (C) levels were calculated as:

$H° = \tan^{-1}\left(\frac{b^*}{a^*}\right) \times 57.29$ (expressed in degrees); $C = \sqrt{(a^{*2} + b^{*2})}$.

The drip loss sample was divided into two equal portions weighed and suspended in an inflated plastic bag. Drip loss was estimated as a percentage ratio of weight loss to initial weight after meat samples were stored for 24 h, at 4 °C in a refrigerator (Honikel, 1998).

Table 6.1 Feed ingredients and chemical composition of experimental diets

Component	Inclusion of grape pomace (g/ kg)					
	0	50	100	150	200	
Ingredients						
Sun-dried Pinotage meal[1]	0	50	100	150	200	
Lucerne meal	200	200	200	200	200	
Soybean hulls	37.6	49.2	48.2	38.6	5.6	
Hominy chop (maize bran)	50	50	50	0	0	
Defatted maize germ	150	150	150	150	130	
Wheat bran middling's	90.9	107	46.8	0	0	
Oat bran	50	3.1	0	0	0	
Maize meal	281	275	292	327	325	
Megalac[2]	0	0	0	4.9	20.7	
Molasses syrup	40	40	40	40	40	
Fish meal[3]	0	0	0	0	3.81	
Lupins	13.3	0	5.97	17.3	0	
Soybean meal[4]	38.7	31.4	33.3	39.7	46.9	
Limestone fine	24.8	24.6	18.8	17.4	13.3	
Salt fine	8.2	4.2	4.4	4.6	4.2	
Toxin binder (Mycosorb A)	1	1	1	1	1	
Mold inhibitor (Technigard)	0.5	0.5	0.5	0.5	0.5	
Sal CURB® S liquid mix[5]	5	5	0	0	0	
Vitamin/ Mineral Premix[6]	7	7	7	7	7	
Dust binder (Dustex)	2	2	2	2	2	
Chemical composition						SEM[12]
DM (g/ kg as-fed basis)	880	879	878	880	881	0.55
Organic matter	910	917	928	922	924	1.14
Crude protein	173	179	179	175	174	1.55
Ether extract	37.1	44.8	49.8	55.0	63.4	1.11
Starch	258	263	261	263	259	7.82
aNDFom[7]	300	301	289	262	243	6.90
ADFom[8]	190	196	166	160	182	3.80
Lignin (sa.) [9]	31.4	47.3	61.0	70.7	77.7	3.71
Metabolizable energy (MJ/ kg DM) [10]	10.9	11.1	11.6	11.5	11.4	0.07
Non-fiber carbohydrates[11]	401	392	410	429	444	7.14
Total tannins (g gallic acid equivalent/ kg DM)	0	27.4	34.7	48.7	59.4	2.20
Proanthocyanidins (g cyanidin chloride equivalent/ kg DM)	0	7.9	12.3	17.8	25.7	0.20

[1] Chemical composition GP meal: 919 g/ kg DM; ash, 56.8 g/ kg DM; crude protein, 107 g/ kg DM; ether extract, 89.3 g/ kg DM; starch, 69.2 g/ kg DM, aNDFom, 401 g/ kg; total tannins, 143 g/ kg DM; proanthocyanidins, 64.9 g/ kg DM.
[2] Megalac: A high-energy rumen-protected fat supplement (Calcium Salt of Palm Fatty Acids)
[3] Fish meal containing 650 g/ kg protein.
[4] Soybean meal: Soybean containing 470 g/ kg protein.
[5] Sal CURB® S liquid mix: antimicrobial used to control *Salmonella* contamination
[6] Vitamin/ mineral premix (i.e., MW Sheep PX with Monesin 68813). The composition of the vitamin/ premix was not included because of a non-disclosure agreement with the feed manufacturer.
[7] aNDFom: neutral detergent fiber assayed with heat stable amylase and expressed exclusive of ash.
[8] ADFom: Acid detergent fiber expressed exclusive of ash.
[9] Lignin (sa): Lignin determined by solubilization of cellulose with sulfuric acid.
[10] Estimated according to CSIRO (2007).
[11] Non-fiber carbohydrates: Calculated as: 1000– (aNDFom g/ kg + crude protein g/ kg + ether extract g/ kg + ash g/ kg).
[12] SEM: Standard error of mean.

6.2.5.2 Meat proximate analyses

The LTL slice for proximate analyses was trimmed of all subcutaneous fat before being homogenized using a knife mill (Knifetec™ 1095, Höganäs., Sweden), vacuum-packed and stored at -20 °C for chemical analyses. Moisture (method 934.01) and ash (method 942.05) were determined according to the (AOAC, 2002). Total fat was extracted using chloroform/ methanol (2/ 1 v/ v) solvent and determined according to Lee et al. (1996). Protein content was determined using DUMAS method (LECO® FP528, LECO Corporation, Miami, USA). Proximate analyses were conducted in duplicate

6.2.5.3 Cooking loss and tenderness

For cooking loss determination, meat samples were weighed, placed in plastic bags and immersed in a water-bath at 80 °C for 60 min to reach an internal temperature of 75 °C monitored using a thermocouple attached to a handheld digital temperature monitor (Hanna Instruments, Bellville, South Africa). The bags were cooled to ± 4 °C and LTL slices were removed and blotted dry with paper towels, without any added pressure and re-weighed. Cooking loss was calculated as the percentage difference in weight before and after cooking (Honikel, 1998). The cooked samples were chilled at 4 °C for 24 h for the determination of Warner-Braztler shear force. A minimum of six cores from each meat sample were removed in the direction of the muscle fibers using a sharp, stainless steel 1.27 cm diameter borer. Core shear force was determined using a V-shaped, 1-mm thick Warner Bratzler cutting blade (speed; 200 mm/ min) attached to an Instron 3345 (Universal) equipped with a 500 N load cell, to determine the shear force in Newtons.

6.2.5.4 Gross profit analyses

A gross profit analysis was conducted to determine the financial viability of incorporating red GP meal in lamb finisher diets. Gross profit was calculated as the difference between total revenue and total variable costs. In the present study, total variable costs were exclusively based on the major contributory factors to expenses that is, cost of purchasing lambs and total feed consumed per lamb. All the other expenses were similar across the different diets, therefore, excluded from the gross profit analyses. Total revenue for each treatment was determined as the income received from the selling of each carcass.

6.2.6 Statistical analyses

Carcass attributes, meat quality traits and gross profit data were analyzed using the mixed model procedure (PROC MIXED) of SAS (2012) with diet as a fixed factor. Animal was used as the experimental unit. Regarding feed intake and ADG, day was added in the model as a repeated measure, with initial weight as a covariate. Treatment means were generated using the LSMEANS option of SAS (2012). A response surface regression (PROC RSREG) analysis of SAS (2012) was conducted to test for linear and quadratic effects of increasing dietary levels of grape pomace on growth, carcass and meat physicochemical attributes and determine the stationary point (plateau) and its critical value (optimum inclusion level). Significance was declared at $P \leq 0.05$ and a tendency at $0.05 < P \leq 0.10$.

6.3 Results

6.3.1 Dietary ingredients and chemical composition

Diets were made isoenergetic and isonitrogenous by substitution of fibrous ingredients with GP. For the 50 and 100 g GP / kg of diets DM, substitutions were mainly for oat bran and wheat bran middlings, and for the 150 and 200 g GP / kg of diets DM, the substitutions were mainly for hominy chop and soybean hulls (Table 6.1).

6.3.2 Growth performance and carcass traits

Table 6.2 shows the effects of increasing dietary levels of red GP on lamb growth and carcass attributes. Initial live weights were not different among diets ($P > 0.05$). Dry matter intake showed a positive quadratic ($P \leq 0.05$) response with incremental levels of GP with a critical value of 113 g GP / kg of diet (Fig. 6.1). Grape pomace inclusion quadratically affected ($P \leq 0.05$) final live weight, wherein the highest gain was achieved at 97 g GP / kg of diet (Fig. 6.1; $P \leq 0.05$). Similarly, ADG exhibited a quadratic response ($P \leq 0.05$) with an optimum inclusion level achieved at 96 g GP / kg of diet of the diet (Fig. 6.1). Feed conversion efficiency neither exhibited a linear nor quadratic response to the diet ($P > 0.05$). Carcass weights (hot and cold) increased in a quadratic fashion ($P \leq 0.05$) with increasing levels of GP reaching a peak at 122 and 121 g GP / kg of diet, respectively (Fig. 6.1). Dressing percentage linearly increased ($P \leq 0.05$) with inclusion of GP diets. The temperature and pH at 45 min and 24 h did not differ significantly ($P > 0.05$) with increasing levels of GP. All the carcasses of lambs fed the control and 150 g GP / kg of diets were lean (fat class 2) with round conformation. Ninety-two percent of the lamb carcasses fed the 50 g

GP / kg of diet were lean and round while 10% were classed medium and round. In the 100 g GP / kg of diet, 75% of the carcasses were lean and round and 25% were classed medium and round. For the 200 g GP / kg of diet, 50% of the carcasses were classified as lean and round, 37.5%, medium and round and 12.5%, fat and round.

Table 6.2 Effects of increasing dietary grape pomace (*Vitis vinifera* L. cv. Pinotage) supplementation on the growth performance and carcass traits of Dohne Merino wether lambs

Item	Inclusion of grape pomace (g/ kg GP)					SEM[a]	P-value		
	0	50	100	150	200		Diet	Linear	Quadratic
Initial live weight, kg	31.2	32.0	32.6	32.2	32.1	0.61	0.596	0.296	0.217
Final live weight, kg	48.6	51.6	52.3	50.3	49.5	0.78	0.012	0.645	0.003
Average daily gain, kg	0.41	0.47	0.47	0.43	0.41	0.012	0.010	0.596	0.003
Dry matter intake, kg/ d	1.61	1.75	1.80	1.71	1.71	0.15	0.001	0.106	0.004
Feed efficiency	0.21	0.22	0.22	0.21	0.20	0.01	0.320	0.118	0.158
Hot carcass weight, kg	23.4	24.7	26.0	24.9	24.9	0.46	0.049	0.061	0.009
Cold carcass weight, kg	22.7	23.9	25.2	24.1	24.1	0.44	0.048	0.063	0.007
Dressing %	48.0	47.9	49.8	49.6	50.2	0.53	0.048	0.068	0.826
pH (45 min)	6.41	6.50	6.55	6.42	6.51	0.09	0.747	0.657	0.799
pH (24 h)	5.78	5.73	5.79	5.78	5.66	0.04	0.403	0.338	0.367
Temperature (45 min)	36.8	37.1	37.2	38.1	36.5	0.50	0.327	0.782	0.229
Temperature (24 h)	6.78	6.82	6.75	6.77	6.79	0.09	0.984	0.923	0.624

[a] SEM: standard error of the mean.
[b] Probability value for linear and quadratic contrasts.

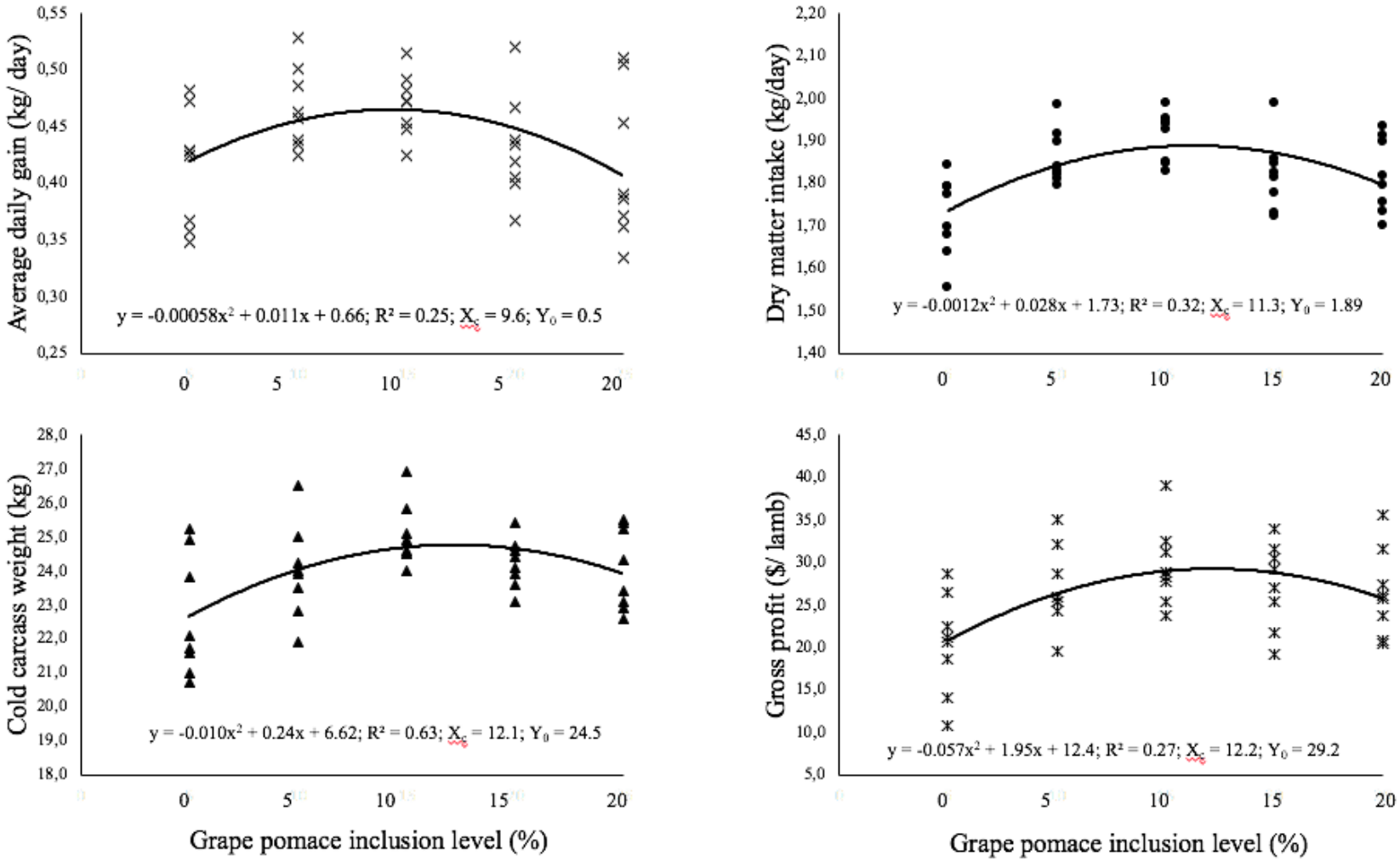


Fig. 6.1 Growth and carcass traits of lambs fed varying levels of red grape pomace with a quadratic function.
Y_0: Plateau - maximum or minimum value for each quadratic parameter.
X_c: Critical value - optimum inclusion level (g/kg) of GP for each quadratic parameter.

6.3.3 Physicochemical meat quality traits

The effects of GP supplementation on meat physical and chemical traits are presented in Table 6.3. Increasing levels of GP in the diet had no effect ($P > 0.05$) on the moisture, ash and intramuscular fat contents of lamb meat. Protein content, however, showed quadratic tendencies ($P \leq 0.1$) with increasing levels of GP. Supplementation with GP had no effect on meat lightness (L*), redness (a*), yellowness (b*) and chroma ($P > 0.05$). Cooking loss exhibited a negative quadratic response ($P \leq 0.05$) with the critical value of 111 g GP / kg of diet, corresponding to the lowest loss. Warner-Bratzler shear force was not influenced ($P > 0.05$) by increasing levels of GP in the diet.

6.3.4 Gross profit analysis

The gross profit analysis for GP supplemented lamb finisher diets are shown in Table 6.4. Total lamb and total variable costs were not affected ($P > 0.05$) by the increase in GP levels. Total feed costs, revenue and gross profit increased quadratically with an increasing level of GP with critical values ranging between 107 and 122 g GP / kg of diet ($P \leq 0.05$).

Table 6.3 Effects of increasing dietary grape pomace (*Vitis vinifera* L. cv. Pinotage) supplementation on the physicochemical quality of *Longissimus thoracis et lumborum* muscle of Dohne Merino wether lambs

Item	Inclusion of grape pomace (g/ kg)					SEM[a]	P-value		
	0	50	100	150	200		Diet	Linear	Quadratic
Moisture, %	73.9	73.8	73.9	74.5	74.5	0.31	0.537	0.161	0.833
Ash, %	1.03	1.13	1.02	0.97	1.14	0.05	0.109	0.436	0.481
Crude protein, %	22.8	22.8	23.4	22.5	22.1	0.33	0.159	0.103	0.091
Intramuscular fat, %	2.27	2.35	2.16	2.33	2.22	0.21	0.965	0.428	0.995
L*	39.0	38.4	38.2	39.7	39.0	0.61	0.468	0.532	0.510
a*	11.0	11.1	11.4	10.9	11.2	0.36	0.900	0.884	0.862
b*	8.95	8.45	8.70	9.07	8.58	0.21	0.124	0.857	0.832
Hue angle (H°)	39.1	37.5	37.4	41.0	37.6	1.05	0.090	0.855	0.967
Chroma (C)	14.2	13.9	14.3	14.4	14.1	0.33	0.869	0.743	0.793
Drip loss, %	1.66	1.47	1.58	1.78	1.38	0.11	0.122	0.520	0.479
Cooking loss, %	40.3	38.1	37.7	37.7	39.3	0.21	0.001	0.110	<0.001
[1]WBSF, N	41.6	47.5	45.0	36.4	45.4	2.95	0.351	0.955	0.764

[a] SEM: standard error of the mean.
[b] Probability value for linear and quadratic contrasts.
[c] WBSF- Warner-Braztler shear force.

Table 6.4 Effects of increasing dietary grape pomace (Vitis vinifera L. cv. Pinotage) supplementation on gross profit (US$/ lamb) of Dohne Merino wether lambs

Variable	Inclusion of grape pomace (g/ kg)					SEM[a]	P-value		
	0	50	100	150	200		Diet	Lin.[b]	Quad.[b]
Total lamb cost	83.9	83.2	85.9	84.6	84.8	1.56	0.753	0.513	0.615
Total feed cost	24.6	25.6	26.3	25.2	25.2	0.42	0.094	0.594	0.019
Total variable cost	109	109	112	110	110	1.69	0.537	0.467	0.291
Total revenue	129	136	141	137	137	2.45	0.013	0.041	0.006
Gross profit	20.3	27.0	29.5	27.3	26.4	1.82	0.014	0.035	0.005

[a] SEM: standard error of the mean.
[b] Probability value for linear and quadratic contrasts.

6.4 Discussion

The high DMI observed up to the 113 g GP / kg of diet inclusion may be attributed to the substitution of oat bran and wheat bran middlings with GP. The reason for this is not known, but perhaps it may be related to improved palatability or perhaps increased rate of passage and reduced gut fill (Makkar, 2003). Beyond the critical level, the reduction in DMI could be partly explained by the increasing phenolic and lignin contents (Calderón-Cortés et al., 2018; Moore and Jung, 2001; Waghorn, 2008). Calderón-Cortés et al. (2018) suggested that the inclusion of dried GP beyond 10% is usually accompanied by a reduction of available energy in lambs, possibly because of the associative effects of polyphenols. Tanniferous diets negatively affect the rate of fiber digestion, thus will slow the clearance of feed residues from the rumen, which may necessitate more rumination and consequently reduce voluntary feed intake, especially at higher tannin concentration (Waghorn, 2008). Additionally, tannins bind with feed proteins, the microflora themselves or microbial enzymes, thereby reducing fiber digestion (Waghorn, 2008), subsequently reduce DMI through rumen fill effect. The reduction in DMI with increasing levels of polyphenolic compounds in the diet is also associated with their astringent taste (Makkar, 2003).

The reduction in DMI beyond the critical level could also be partially attributed to the increasing lignin content. Lignin acts as a physical barrier to microbial enzymes reaching their target polysaccharides (Moore and Jung, 2001). Thus, the higher the lignin content, the less DM consumed by an animal (Mertens, 1994; Van Soest, 1994), which can explain the decline of DMI once the peak level is reached. This results in the undigested portion of the fiber being retained longer in the rumen because of reduced digestibility and contributes to the fill effect of the diet (Moore and Jung, 2001), thereby reducing DMI. Abarghuei et al. (2010) observed that high lignin content in sheep diets was the major cause of decreased digestibility of DM and NDF. Overall, the quadratic trend in DMI observed when feeding increasing levels of GP could be attributed to a

combination of the nutritive profile of the feed (Alonso-Díaz et al., 2010), particularly, the content of lignin and tannins (Tedeschi et al., 2014). Despite the DMI values in the current study being slightly higher than ram lambs fed GP (Zhao et al., 2018), they are within the ranges observed by Brand et al. (2017) in weaner lamb breeds on a commercial feedlot diet.

The quadratic pattern for ADG and final live weight with addition of GP is related to changes in DMI, and the combined effects of high crude protein and moderate proanthocyanidin contents reported for the 50 and 100 g/ kg GP diets. On one hand, moderate to low proanthocyanidin content (<50 g/ kg DM) can either bind directly with dietary proteins in the rumen or to microbial enzymes and/ or inhibit growth of rumen microbes involved in protein degradation, with subsequently higher flow of essential amino acids to the duodenum (Mueller-Harvey, 2006; Waghorn et al., 1994). These complexes improve protein availability post-ruminally and increase weight gain in growing lambs as was observed by Zhao et al. (2018). On the other hand, high levels of GP proanthocyanidins (>50 g/ kg DM), both *in vivo* and *in vitro* have been reported to decrease protein degradability in the rumen (Abarghuei et al., 2010; Basalan et al., 2011), with subsequent reductions in flow of essential amino acids to duodenum, and weight gains. Although, the current treatments did not surpass the 50 g proanthocyanidin/ kg DM, Mueller-Harvey (2006) alludes that the effect of tannins does not only depend on the content but also the source and structure.

The quadratic responses to GP addition reported for hot and cold carcass weights are consistent with differences found in DMI, final live weight and ADG. It is expected that if animals have higher DMI and ADG, they tend to have greater muscle and fat deposition, and consequently high slaughter weights and heavier carcasses (Mapiye et al., 2009). The opposite is true for animals with lower DMI and ADG. The lack of a significant effect of dietary tannin containing-feed on meat ultimate pH is not uncommon (Zhao et al., 2017; Zhao et al., 2018). The present values are within the acceptable ultimate pH range (5.6 – 5.8) for ovine carcasses (Jiang et al., 2014;

Majdoub-Mathlouthi et al., 2013) and further rule out the formation of dark, firm and dry meat (i.e., dark cutting) or stress problems among the lambs (Smeti et al., 2013).

The lack of color differences between the control and phenolic-containing diets 24 h postmortem is consistent with other studies on wine GP (Zhao et al., 2018) and grape seed extract (Jerónimo et al., 2012). The redness values observed in the current study (10.9 – 11.4) exceeds the threshold value of ≥ 9.5, which consumers consider acceptable lamb meat color (Khliji et al., 2010). Zhao et al. (2017) ascribes the lack of color differences in lamb meat compared to other species like pork regardless of diet, to an overall darker pigmentation, therefore, masking any color improvement. The high protein content in GP finished lamb meat, more specifically, the 10% inclusion level might be attributed to individual or combined effects of moderate tannin and high protein contents in the diet as explained previously. This phenomenon was reinforced by Orlandi et al. (2015) who observed closely related amino acid profiles between dietary and duodenal digesta of steers fed increasing rate of *Acacia mearnsii* tannin extract.

There is no clear explanation for the observed effect of diet on the cooking loss. The differences in the cooking loss among the dietary treatments are of low magnitude over a practical point of view. Differences could have arisen due to other factors such as cooking methods, temperature, heating rates and endpoint center temperatures (Lopes et al., 2014). Researchers usually disregard other important variables such as diet composition (digestible carbohydrates, magnesium, vitamin E and D3), muscle fiber length, sample size and dimension, meat cooling temperature and rate (Jacques et al., 2017). These factors may be influential and be used to explain some of the variation in cooking loss, but unfortunately these details were also not evaluated in the current study. The lack of dietary influence on WBSF concurs with previous findings by Zhao et al. (2018) who fed tannin-rich diets. Nevertheless, the shear force values were below 49 N, a

threshold that has been set as an indication of acceptable tenderness in sheep meat (Hopkins et al., 2006).

The quadratic response observed for gross profit with increasing GP in lamb diets could be attributed to a similar pattern for DMI and subsequently higher ADG and carcass weights reported earlier. Furthermore, the low cost of GP (Manso et al., 2016) contributed to the improved gross profit. Finishing lambs in feedlots is a common practice that has been increasing over the years, mainly on an opportunity basis to increase the gross profit of lamb finishing when lamb meat prices are high (Jolly and Cottle, 2010). The current findings suggest substituting fiber sources in lamb finishing diets with red GP, rich in polyphenolic compounds, has added advantage for reducing feed costs and at the same time improving growth performance and carcass traits, without compromising meat quality. Hence, GP can be used to partially substitute commonly used fiber sources without compromising production, provided dietary formulations are isoenergetic and isonitrogenous.

6.5 Conclusions

Based on the current findings, GP may be adopted as a feed ingredient in lamb diets at between 100 and 120 g GP / kg of diet DM as a strategy to improve production and decrease feeding costs. Additionally, it could reduce economic and environmental costs associated with disposing or recycling winery wastes, especially in areas where GP is abundant. Further studies should be conducted to assess the effects of graded levels of sun-dried GP on the shelf life, fatty acid profile and sensory quality of lamb meat.

6.6 References

Abarghuei, M.J., Rouzbehan, Y., Alipour, D., 2010. The influence of the grape pomace on the ruminal parameters of sheep. Livest. Sci. 132, 73–79.

Alonso-Díaz, M.A., Torres-Acosta, J.F.J., Sandoval-Castro, C.A., Hoste, H., 2010. Tannins in tropical tree fodders fed to small ruminants: A friendly foe? Small Rumin. Res. 89, 164–173.

AOAC (Association of Official Analytical Chemists), 2002. Official Methods of Analysis, 17th ed. Association of Official Analytical Chemists Inc., Arlington, Virginia, USA.

Basalan, M., Gungor, T., Owens, F.N., Yalcinkaya, I., 2011. Nutrient content and in vitro digestibility of Turkish grape pomaces. Anim. Feed Sci. Technol. 169, 194–198.

Baumgärtel, T., Kluth, H., Epperlein, K., Rodehutscord, M., 2007. A note on digestibility and energy value for sheep of different grape pomace. Small Rumin. Res. 67, 302–306.

Beres, C., Costa, G.N.S., Cabezudo, I., da Silva-James, N.K., Teles, A.S.C., Cruz, A.P.G., Mellinger-Silva, C., Tonon, R. V., Cabral, L.M.C., Freitas, S.P., 2017. Towards integral utilization of grape pomace from winemaking process: A review. Waste Manag. 68, 581–594.

Brand, T.S., Van Der Westhuizen, E.J., Van Der Merwe, D.A., Hoffman, L.C., 2017. Effect of days in feedlot on growth performance and carcass characteristics of Merino, South African Mutton Merino and Dorper lambs. S. Afr. J. Anim. Sci. 47, 26–33.

Brenes, A., Viveros, A., Chamorro, S., Arija, I., 2016. Use of polyphenol-rich grape byproducts in monogastric nutrition. A review, Animal Feed Science and Technology. 211, 1-17.

Calderón-Cortés, J.F., González-Vizcarra, V.M., Pétriz-Celaya, Y., Pujol, L.C., Barreras, A., Plascencia, A., 2018. Energy value of unfermented dried grape pomace as substitute of alfalfa hay in diets for growing lambs. Aust. J. Vet. Sci. 63, 59–63.

Chikwanha, O.C., Raffrenato, E., Muchenje, V., Musarurwa, H.T., Mapiye, C., 2018. Varietal differences in nutrient, amino acid and mineral composition and in vitro rumen digestibility of grape (*Vitis vinifera*) pomace from the Cape Winelands vineyards in South Africa and impact of preservation techniques. Ind. Crops Prod. 118, 30–37.

CSIRO (Commonwealth Scientific and Industrial Research Organization), 2007. Nutrient Requirements of Domesticated Ruminants. CSIRO Publishing, Collingwood VIC 3066, Australia.

Dwyer, K., Hosseinian, F., Rod, M., 2014. The market potential of grape waste alternatives. J. Food Res. 3, 91–106.

García-Lomillo, J., González-SanJosé, M.L., 2017. Applications of wine pomace in the food industry: Approaches and functions. Compr. Rev. Food Sci. Food Saf. 16, 3–22.

Goering, H.K., Van Soest, P.J., 1970. Forage Fiber Analyses (Apparatus, Reagents, Procedures and some Applications) - Agricultural handbook no.379. US Dept. of Agriculture, Washington DC., USA.

Guerra-Rivas, C., Gallardo, B., Mantecón, Á.R., del Álamo-Sanza, M., Manso, T., 2017. Evaluation of grape pomace from red wine byproduct as feed for sheep. J. Sci. Food Agric. 97, 1885–1893.

Hall, M.B., 2009. Determination of starch, including maltooligosaccharides, in animal feeds: Comparison of methods and a method recommended for AOAC collaborative study. J. AOAC Int. 92, 42–49.

Honikel, K.O., 1998. Reference methods for the assessment of physical characteristics of meat. Meat Sci. 49, 447–457.

Hopkins, D.L., Hegarty, R.S., Walker, P.J., Pethick, D.W., 2006. Relationship between animal age, intramuscular fat, cooking loss, pH, shear force and eating quality of aged meat from sheep. Aust. J. Exp. Agric. 46, 879–884.

Jacques, J., Chouinard, Y., Gariépy, C., Cinq-Mars, D., 2016. Meat quality, organoleptic characteristics and fatty acid composition of Dorset lambs fed different forage to concentrate ratio or fresh grass. Can. J. Anim. Sci. 97, C.

Jerónimo, E., Alfaia, C.M.M., Alves, S.P., Dentinho, M.T.P., Prates, J.A.M., Vasta, V., Santos-Silva, J., Bessa, R.J.B., 2012. Effect of dietary grape seed extract and *Cistus ladanifer* L. in combination with vegetable oil supplementation on lamb meat quality. Meat Sci. 92, 841–847.

Jiang, H., Wang, Z., Ma, Y., Qu, Y., Lu, X., Guo, H., Luo, H., 2014. Effect of dietary lycopene supplementation on growth performance, meat quality, fatty acid profile and meat lipid oxidation in lambs in summer conditions. Small Rumin. Res. 131, 99–106.

Jolly, S., Cottle, D.J., 2010. Feeding, in: Cottle, D.J., Melorose, J., Perroy, R., Careas, S. (Eds.), International Sheep and Wool Handbook. Nottingham University Press, Nottingham, pp. 295–300.

Kumar, R., D'mello, J.P.F., Devendra, C., 1995. Anti-nutritional Factors in Forage Legumes. Trop. Legum. Anim. Nutr. 95–133.

Lee, C.M., Trevino, B. and Chaiyawat, M., 1996. A simple and rapid solvent extraction method for determining total lipids in fish tissue. J. Assoc. Anal. Chem. Int. 79, 487–492.

Lopes, L.S., Martins, S.R., Chizzotti, M.L., Busato, K.C., Oliveira, I.M., Machado Neto, O.R., Paulino, P.V.R., Lanna, D.P.D., Ladeira, M.M., 2014. Meat quality and fatty acid profile of Brazilian goats subjected to different nutritional treatments. Meat Sci. 97, 602–608.

Majdoub-Mathlouthi, L., Saïd, B., Say, A., Kraiem, K., 2013. Effect of concentrate level and slaughter body weight on growth performances, carcass traits and meat quality of Barbarine lambs fed oat hay-based diet. Meat Sci. 93, 557–563.

Makkar, H.P.S., 2003. Effects and fate of tannins in ruminant animals, adaptation to tannins, and strategies to overcome detrimental effects of feeding tannin-rich feeds. Small Rumin. Res. 49, 241–256.

Makkar, H.P.S., Siddhuraju, P., Becker, K., 2007. Tannins, in: Makkar, H.P.S., Siddhuraju, P., Becker, K. (Eds.), Plant Secondary Metabolites. Humana Pres Inc., New Jersey, USA, pp. 67–82.

Manso, T., Gallardo, B., Guerra-Rivas, C., 2016. Modifying milk and meat fat quality through feed changes. Small Rumin. Res. 142, 31–37.

Mapiye, C., Chimonyo, M., Dzama, K., Strydom, P.E., Muchenje, V., Marufu, M.C., 2009. Nutritional status, growth performance and carcass characteristics of Nguni steers supplemented with *Acacia karroo* leaf-meal. Livest. Sci. 126, 206–214.

Mertens, D.R., 1994. Regulation of forage Intake, in: Forage Quality, Evaluation, and Utilization. American Society of Agronomy, Crop Science Society of America, Soil Science Society of America, Madison, WI, pp. 494–532.

Mertens, D.R., Allen, M., Carmany, J., Clegg, J., Davidowicz, A., Drouches, M., Frank, K., Gambin, D., Garkie, M., Gildemeister, B., Jeffress, D., Jeon, C.S., Jones, D., Kaplan, D., Kim, G.N., Kobata, S., Main, D., Moua, X., Paul, B., Robertson, J., Taysom, D., Thiex, N., Williams, J., Wolf, M., 2002. Gravimetric determination of amylase-treated neutral detergent fiber in feeds with refluxing in beakers or crucibles: Collaborative study. J. AOAC Int. 85, 1217–1240.

Moate, P.J., Williams, S.R.O., Torok, V.A., Hannah, M.C., Ribaux, B.E., Tavendale, M.H., Eckard, R.J., Jacobs, J.L., Auldist, M.J., Wales, W.J., 2014. Grape marc reduces methane emissions when fed to dairy cows. J. Dairy Sci. 97, 5073–5087.

Moore, K.J., Jung, H.-J.G., 2001. Lignin and fiber digestion. J. Range Manag. 54, 420–430.

Mueller-Harvey, I., 2006. Unravelling the conundrum of tannins in animal nutrition and health. J. Sci. Food Agric. 86, 2010–2037.

Negro, C., Tommasi, L., Miceli, A., 2003. Phenolic compounds and antioxidant activity from red grape marc extracts. Bioresour. Technol. 87, 41–44.

Nkosi, B.D., Meeske, R., 2010. Effects of ensiling totally mixed potato hash ration with or without a heterofermentative bacterial inoculant on silage fermentation, aerobic stability, growth performance and digestibility in lambs. Anim. Feed Sci. Technol. 161, 38–48.

National Research Council, 2007. Nutrient Requirements of Small Ruminants. National Academy Press, Washington DC, USA.

Orlandi, T., Kozloski, G. V., Alves, T.P., Mesquita, F.R., Ávila, S.C., 2015. Digestibility, ruminal fermentation and duodenal flux of amino acids in steers fed grass forage plus concentrate containing increasing levels of *Acacia mearnsii* tannin extract. Anim. Feed Sci. Technol. 210, 37–45.

Perez-Maldonado, R.A., Norton, B.W., 1996. Digestion of 14C-labelled condensed tannins from Desmodium intortum in sheep and goats. Br. J. Nutr. 76, 501–513.

Pirmohammadi, R., Golgasemgarebagh, A., Azari, A.M., 2007. Effects of ensiling and drying of white grape pomace on chemical composition, degradability and digestibility for ruminants. J. Anim. Vet. Adv. 6, 1107-1111.

Porter, L.J., Hrstich, L.N., Chan, B.G., 1986. The conversion of procyanidins and prodelphinidins to cyanidin and delphinidin. Phytochemistry 25, 223–230.

Raffrenato, E., Van Amburgh, M.E., 2011. Technical note: Improved methodology for analyses of acid detergent fiber and acid detergent lignin. J. Dairy Sci. 94, 3613–3617.

Smeti, S., Atti, N., Mahouachi, M., Munoz, F., 2013. Use of dietary rosemary (*Rosmarinus officinalis* L.) essential oils to increase the shelf life of Barbarine light lamb meat. Small Rumin. Res. 113, 340–345.

South African Meat Industry Company, 2006. Classification of red meat. http://www.samic.co.za/downloads/Redmeat.pdf. (accessed 3.14.18).

Tedeschi, L.O., Ramírez-Restrepo, C.A., Muir, J.P., 2014. Developing a conceptual model of possible benefits of condensed tannins for ruminant production. Animal 8, 1095–1105.

Van Soest, P.J., 1994. Nutritional Ecology of the Ruminant, 2nd ed. Cornell University Press, Ithaca, USA.

Waghorn, G., 2008. Beneficial and detrimental effects of dietary condensed tannins for sustainable sheep and goat production-Progress and challenges. Anim. Feed Sci. Technol. 147, 116–139.

Waghorn, G.C., Shelton, I.D., McNabb, W.C., McCutcheon, S.N., 1994. Effects of condensed tannins in *Lotus penduculatus* on its nutritive value for sheep. 2. Nitrogenous aspects. J. Agric. Sci. 123, 109–119.

Zepf, F., Jin, B., 2013. Bioconversion of grape marc into protein rich animal feed by microbial fungi. Chem. Eng. Process Tech. 1, 1011.

Zhang, N., Hoadley, A., Patel, J., Lim, S., Li, C., 2017. Sustainable options for the utilization of solid residues from wine production. Waste Manag. 60, 173–183.

Zhao, J., Li, K., Su, R., Liu, W., Ren, Y., Zhang, C., Du, M., Zhang, J., 2017. Effect of dietary Tartary buckwheat extract supplementation on growth performance, meat quality and antioxidant activity in ewe lambs. Meat Sci. 134, 79–85.

Zhao, J.X., Li, Q., Zhang, R.X., Liu, W.Z., Ren, Y.S., Zhang, C.X., Zhang, J.X., 2018. Effect of dietary grape pomace on growth performance, meat quality and antioxidant activity in ram lambs. Anim. Feed Sci. Technol. 236, 76–85.

Chapter 7 Shelf life stability and eating quality of grape pomace-enhanced lamb meat[6]

ABSTRACT

The current study evaluated eating quality, oxidative and microbial stability of meat from lambs fed finisher diets containing increasing levels of grape pomace (GP). Antioxidant capacity, color and lipid oxidation were analyzed on diets containing 0, 5, 10, 15 and 20% GP/ kg on d 1, 3, 5, 7 and 9. Protein oxidation and microbial analyses were performed on diets containing 0, 10 and 20% GP/ kg on d 1, 5 and 9, while sensory quality was evaluated on the same diets on d 1. Diet had no effect on meat color, microbial load and sensory quality ($P > 0.05$). Diet × day interactions were observed for antioxidant activity, lipid and protein oxidation ($P \leq 0.05$). Overall, the 20% GP-diet finished lamb meat had the highest antioxidant power, the lowest lipid and protein oxidation values during retail display ($P \leq 0.05$). Inclusion of GP in lamb diets improved the meat oxidative stability without negatively eating quality.

Key words

[6] A version of this chapter was submitted for publication:
Shelf life stability and eating quality of grape (*Vitis vinifera* L. cv. Pinotage) pomace-enhanced lamb meat. Meat Science

Lamb meat, Microbial growth, Oxidative stability, Sensory quality, Shelf life, Winery waste

7.1 Introduction

7.2 Materials and methods

An estimated 20% of the 263 million tons of meat produced globally is lost or wasted (FAO, 2019). This represents a missed opportunity to improve global food security, especially in the developing countries where protein intake level is low (Gustavsson et al., 2011). Oxidative deterioration of biomolecules and microbial spoilage represents the major factors responsible for meat losses and waste (Cunha et al., 2018; Falowo et al., 2014; Bekhit et al., 2013; Papuc et al., 2017). Lipid oxidation is the primary cause of quality deterioration in meat (Kumar et al., 2015). This occurs concurrently with the oxidation of heme pigments, but the latter have received less attention in meat systems compared to that of lipids (Estévez and Luna, 2017; Soladoye et al., 2015). Yet all these processes adversely affect the nutritional quality, shelf life and sensory quality of meat, which negatively impact on meat consumption (Cunha et al., 2018; Papuc et al., 2017). Oxidation is more pronounced under retail commercial conditions where the shelf life for air-permeable over-wrap meat is between two to five days (Warner et al., 2017).

In addition to oxidation, microbial contamination also contributes to meat spoilage, and both processes subsequently reduce shelf life (Papuc et al., 2017). The spoilage of meat accounts for over 40% of total production losses from slaughter to the retail market (Lahmar et al., 2018). Food-borne pathogens are the leading cause of illness and death in developing countries (Praet et al., 2015).

Currently, synthetics are the dominant preservatives used in animal nutrition and the meat industry to counteract the effect of oxidation and microbial spoilage (Papuc et al., 2017; Sun and

Holley, 2012). These synthetics have serious impact on human health as their long-term use has been linked to liver damage, asthma, allergic reactions and cancer (Bedale et al., 2016; Mattos et al., 2017; Ribeiro et al., 2019). This has shaped meat consumers' attitude towards trust and preference for natural preservatives. Natural preservatives are perceived to be nutritious, safe, healthful and sustainable (Chibane et al., 2019; Ribeiro et al., 2019). In response, researchers have embarked on finding sustainable natural preservatives with similar or better efficacy than synthetic ones (Cunha et al., 2018; Mattos et al., 2017). Recent research shows that inclusion of natural preservatives in animal diets extends shelf life of meat during storage (Kafantaris et al., 2018; Manso et al., 2016). Subsequently, there has been a general increase in the valorization of fruit by-products because they contain bioactive compounds including vitamins A and E, carotenoids and phenolic compounds, which have antioxidant and antibacterial properties (Andrés et al., 2017; Guerra-Rivas et al., 2016).

Grains (starch-rich) and oilseed cakes (PUFA-rich) are the most common feed ingredients for feedlot finisher lambs in South Africa (Brand et al., 2017) and the world at large (Eisler et al., 2014). Meat from animals fed such diets is more susceptible to oxidation as opposed to that from grass-fed animals (Luciano et al., 2009), as the latter is endowed with natural antioxidants (Descalzo et al., 2005). Co-feeding high-PUFA diets with antioxidants, not only protects PUFA from biohydrogenation in the rumen, but also confers protection against meat macromolecule oxidation and/ or reduces the extent of microbial spoilage during retail shelf display (Cunha et al., 2018; Gravador et al., 2015; Papuc et al., 2017). Dietary antioxidants have been proven to be a simple, convenient and more effective strategy to stabilize components involved in oxidation processes (Bañón et al., 2012; Muela et al., 2014). This is because these compounds are uniformly incorporated into the phospholipid membranes augmenting the existing endogenous antioxidant defense mechanisms as opposed to the direct application onto fresh meat (Bañón et al., 2012;

Muela et al., 2014). Apart from the preservative ability, natural antioxidants have health-promoting properties such as prevention of cancer and cardiovascular diseases (Ávila-Gálvez et al., 2019; Loureiro & Martel, 2019).

In South Africa, winery residues such as grape pomace (GP) have been underexploited by the local meat industry as a source of antioxidant and/ or antimicrobials (Chikwanha et al., 2019). This is despite the high levels and diverse phenolic compounds retained after the pressing of grape berries during wine production (García-Lomillo and González-SanJosé, 2017). To date, no study in South Africa has investigated the keeping and eating qualities of meat from ruminants fed local GP varieties. The objectives of this study was, therefore, to evaluate the effect of feeding finisher diets containing increasing levels of sun-dried red grape (Vitis vinifera L. cv. Pinotage) pomace on the shelf life and sensory quality of lamb meat.

7.2.1 Grape pomace and dietary preparations

Fresh red GP, Pinotage variety was sourced from Bellevue Wine Estate (33.879866° S, 18.763768° E; Stellenbosch, South Africa). The pomace was immediately sun-dried after grape berry pressing. The pomace was dried on a 6 mm polyethylene canvas sheets for 7 days to ensure a moisture content of less than 10% (Chikwanha et al., 2018). The dried pomace was milled (Drotsky M16 Hammer mill, Aktief (Pty) Ltd, Johannesburg, South Africa) to pass through a 4 mm sieve and used as a fibrous ingredient in lamb finisher diets. Five diets were pelleted at a temperature of 80 °C by a commercial feed manufacturing company (Table 7.1). The pellet diameter and average length were 5 mm and ± 30 mm length, respectively. The diets were formulated to be isoenergetic and isonitrogenous by substitution of fibrous ingredients with GP (National Research Council, 2007). Overall, substitutions in the diets were mainly for wheat bran middlings, oat bran and soybean hulls (Table 7.1).

7.2.2 Treatments and animal management

The rearing and slaughter of the animals were described in a companion paper (Chikwanha et al., 2019). Briefly, 40 Dohne merino lambs averaging 32 ± 1.7 kg (~3 – 4 months old) were randomly assigned to five dietary treatments containing 0, 5, 10, 15 and 20% GP/ kg of diet (n = 8) in a completely randomized design. Lambs were housed individually in slated floor pens (2 m^2). Data collection commenced after a 14-day adaptation period and a growth trial which lasted for 42 days were feed intake and performance were monitored.

7.3 Slaughter and meat sampling

At the end of the feeding trial, the lambs were transported to a commercial abattoir 70 km from the experimental site. Lambs were electrically stunned (200 V applied for 4 seconds) and slaughtered by exsanguination after 16 h off feed, with access to water only. Twenty-four hours postmortem, the *longissimus thoracis et lumborum* (LTL) muscles from both sides of the carcass were cut. The right LTL was vacuum packed and stored at -20 °C until sensory evaluation on three treatments (0, 10, and 20 % GP/ kg diet DM) (n = 8). The left *longissimus lumborum* (LL) was used for the shelf life study. For antioxidant activity, lipid and protein oxidation analyses, all subcutaneous fat was trimmed off from each sample before homogenization using a knife mill (Knifetec™ 1095, Höganäs., Sweden) and stored at -80 °C pending analyses. Five portions of 2 cm were aseptically cut with sterile knives and cutting boards and each placed on a square-cut stomacher bag (6 cm × 6 cm) and randomly assigned to five white polystyrene trays (d 1, 3, 5, 7 or 9). The trays were covered by a 10 μm thick oxygen permeable cling film (Versafilm, Crown National, Montague Gardens, Cape Town, South Africa) with a moisture vapor transfer rate of 585 g/ (cm^2 × 24 h × 1 atmosphere), oxygen permeability of 25 000 cm^3/ (m^2 × 24 h × 1 atmosphere) and a carbon dioxide permeability of 180 000 cm^3/ (m^2 × 24 h ×1 atmosphere). All trays were

displayed under continuous, cool and white fluorescent illumination (Philips TL-D 58W/ 33-640 1SL/ 25, Bielsko-Biała, Poland) for a 9-day shelf life study at 4 °C simulating retail display conditions. Every 24 h, trays were rotated to minimize temperature variation and effects due to the differences in light intensities. Color, antioxidant activity and lipid oxidation of meat was conducted on all the five-time periods across all treatments (i.e., 0, 5, 10, 15 and 20 % GP/ kg diet DM; n = 8), while protein oxidation and microbial growth analyses were conducted on three-time periods (i.e., d 1, 5 and 7) and on three treatments (i.e., 0, 10, and 20 % GP/ kg diet DM) (n = 5). On sampling days, a portion of meat for microbial analyses was aseptically cut before color measurements and stored at -20 °C prior to analyses.

7.3.1 Experimental designs, slaughter and meat sampling

Forty castrated Dohne Merino lambs averaging 32 ± 1.7 kg and 3 - 4 months old were purchased from a commercial feedlot in Hermon, South Africa. All the animals were treated for external (Inverject, FarmVet, South Africa) and internal (ByBoost Lamb and Kid + copper, Bayer (Pty) Ltd., South Africa) parasites, and vaccinated against enterotoxaemia and Pasteurella (Enteroprotect P 100, DP, South Africa). Lambs were housed individually in slated floor pens (2 m^2). Lambs were assigned to dietary treatments (8 lambs/ treatment) in a completely randomized design. Data collection commenced after a 14-day adaptation period and lasted for 42 days during which growth performance was monitored. At the end of the feeding trial, the lambs were transported to a commercial abattoir 70 km from the experimental site. Lambs were electrically stunned (200 V applied for 4 seconds) and slaughtered by exsanguination after 16 h off feed, with access to water only.

Twenty-four hours postmortem, the *longissimus thoracis et lumborum* (LTL) muscles from both sides of the carcass were cut. The right LTL was vacuum packed and stored at -20 °C until sensory evaluation (0, 100, and 200 g GP / kg of diet DM) (n = 8). The left LTL was aseptically

sliced (2 cm thick steaks) using sterile knives and cutting boards. Antioxidant activity and lipid oxidation of meat was conducted for all the days for all five treatments, while protein oxidation and microbial status was conducted on days 1, 5 and 7 and on three treatments 0, 100 and g GP / kg of diet DM. One steak per treatment (n = 8) was placed on top of sterile square-cut stomacher bags (6 cm × 6 cm) and randomly assigned to five white polystyrene trays (1, 3, 5, 7 or 9 d of storage) and covered by a 10 μm thick oxygen permeable cling film (Versafilm, Crown National, Montague Gardens, Cape Town, South Africa) with a moisture vapor transfer rate of 585 g/ (cm^2 × 24 h × 1 atm), oxygen permeability of 25 000 cm^3/ (m^2 × 24 h × 1 atmosphere) and a carbon dioxide permeability of 180 000 cm^3/ (m^2 × 24 h ×1 atm). All trays were displayed under continuous, cool and white fluorescent illumination (Philips TL-D 58W/ 33-640 1SL/ 25, Bielsko-Biała, Poland) for a 9-day shelf life study at 4 °C simulating retail display conditions. Every 24 h, trays were rotated to minimize temperature variation and effects due to the differences in light intensities. On sampling days, a portion of meat for microbial analyses was aseptically cut (before color measurements) and stored at -20 °C. Antioxidant activity, lipid and protein oxidation samples were also cut and stored at -80 °C pending analyses.

Table 7.1 Feed ingredients and chemical composition of experimental diets

Component	Inclusion of grape pomace (g/ kg)				
	0	50	100	150	200
Ingredients					
Sun-dried Pinotage meal[1]	0	50	100	150	200
Lucerne meal	200	200	200	200	200
Soybean hulls	37.6	49.2	48.2	38.6	5.6
Hominy chop (maize bran)	50	50	50	0	0
Defatted maize germ	150	150	150	150	130
Wheat bran middling's	90.9	107	46.8	0	0
Oat bran	50	3.1	0	0	0
Maize meal	281	275	292	327	325
Megalac[2]	0	0	0	4.9	20.7
Molasses syrup	40	40	40	40	40
Fish meal[3]	0	0	0	0	3.81
Lupins	13.3	0	5.97	17.3	0
Soybean meal[4]	38.7	31.4	33.3	39.7	46.9
Limestone fine	24.8	24.6	18.8	17.4	13.3
Salt fine	8.2	4.2	4.4	4.6	4.2
Toxin binder (Mycosorb A)	1	1	1	1	1
Mold inhibitor (Technigard)	0.5	0.5	0.5	0.5	0.5
Sal CURB® S liquid mix[5]	5	5	0	0	0
Vitamin/ Mineral Premix[6]	7	7	7	7	7
Dust binder (Dustex)	2	2	2	2	2
Chemical composition					SEM[12]
DM (g/ kg as-fed basis)	880	879	878	880	881	0.55
Organic matter	910	917	928	922	924	1.14
Crude protein	173	179	179	175	174	1.55
Ether extract	37.1	44.8	49.8	55.0	63.4	1.11
Starch	258	263	261	263	259	7.82
aNDFom[7]	300	301	289	262	243	6.90
ADFom[8]	190	196	166	160	182	3.80
ADL[9]	31.4	47.3	61.0	70.7	77.7	3.71
Metabolizable energy (MJ/ kg DM)[10]	10.9	11.1	11.6	11.5	11.4	0.07
Non-fiber carbohydrates[11]	401	392	410	429	444	7.14
Total tannins (g gallic acid equivalent/ kg DM)	0	27.4	34.7	48.7	59.4	2.20
Proanthocyanidins (g cyanidin chloride equivalent/ kg DM)	0	7.9	12.3	17.8	25.7	0.20

Chemical composition GP meal: 919 g/ kg DM; crude protein, 107 g/ kg DM; ether extract, 89.3 g/ kg DM; starch, 69.2 g/ kg DM, aNDFom, 401 g/ kg; ADFom, 348 g/ kg DM; ADL, 215 g/ kg DM; total tannins, 143 g/ kg DM; proanthocyanidins, 64.9 g/ kg DM.

[2] Megalac: A high-energy rumen-protected fat supplement (Calcium Salt of Palm Fatty Acids)

[3] Fish meal containing 650 g/ kg protein.

[4] Soybean meal: Soybean containing 470 g/ kg protein.

[5] Sal CURB® S liquid mix: antimicrobial used to control *Salmonella* contamination

[6]Vitamin/ mineral premix (i.e., MW Sheep PX with Monesin 68813). The composition of the vitamin/ premix was not included because of a non-disclosure agreement with the feed manufacturer.

[7] aNDFom: neutral detergent fiber assayed with heat stable amylase and expressed exclusive of ash.

[8] ADFom: Acid detergent fiber expressed exclusive of ash.

[9]ADL: Acid detergent lignin.

[10] Estimated according to CSIRO (2007).

[11]Non-fiber carbohydrates: Calculated as: 1000– (aNDFom g/ kg + crude protein g/ kg + ether extract g/ kg + ash g/ kg).

[12] SEM: Standard error of mean.

7.4 Shelf life of retail displayed grape pomace-enhanced lamb meat

7.4.1 Meat color

Color measurements were performed directly on the meat surface. Lightness (L^*), redness (a^*) and yellowness (b^*) parameters were recorded using a color-guide 45°/ 0° colorimeter (BYK-Gardner GmbH, Gerestried, Germany). The aperture diameter was 11 mm and set at illuminant D-65 and viewing angle 10°. For each sample, L^*, a^* and b^* measurements were recorded in triplicate at different points.

7.4.2 Antioxidant activity, lipid and protein oxidation

Antioxidant activity (ferric reducing antioxidant power; FRAP assay) was determined as described by Descalzo et al. (2007). Descalzo, Rossetti, Grigioni, Irurueta, Sancho, Carrete et al. (2007). One-gram sample (in duplicate) of meat was disrupted with 5 mL of potassium phosphate buffer (pH 7.2) for 2 min at 3000 rpm with an IKA® T18 digital Ultra-Turrax® (IKA-Werke GmgH & Co., Germany) disperser. The homogenate was centrifuged at 4024 × g for 30 min before addition of FRAP reagent based on a ratio of 10:1:1 (v/ v/ v) of acetate buffer, 2,4,6-Tris(2-pyridyl)-*s*-triazine and iron (III) chloride, respectively. The mixture was shaken for 3 sec and incubated at 37 °C for 10 min before absorbance was read using a microplate reader (SPECTROstar Nano, BMG, Ortenberg, Germany) at 593 nm. Analyses was performed in duplicate using ferrous sulfate ($Fe_2SO_4 \cdot 7H_2O$, Sigma-Aldrich, Germany) for the standard curve and results were expressed as Fe^{2+} equivalents in µmol.

Thiobarbituric acid reactive substances (TBARS) was used for the determination of lipid oxidation of meat (Gatellier et al., 2005). One-gram sample (in duplicate) of the tissue was homogenized with 10 mL of 0.15M potassium chloride and butylated hydroxytoluene mixture for 20 sec at 3000 rpm with an IKA® T18 digital Ultra-Turrax® (IKA-Werke GmgH & Co., Germany)

disperser. A homogenate 0.5 mL was mixed with 0.25 mL of 1% 2-thiobarbituric acid (w/ v) and 50 mM sodium hydroxide solution. The mixture was kept at 95 °C for 30 min. After cooling, 2 mL of n-butanol was added and vortexed. The mixture was centrifuged for 30 min at 2575 × g. Absorbance was read using a microplate reader (SPECTROstar Nano, BMG, Ortenberg, Germany) at 532 nm and results were expressed as mg malondialdehyde (MDA)/ kg meat.

Protein oxidation was determined by measuring the carbonyl content following the colorimetric protocol detailed in the Protein Carbonyl Assay Kit technical bulletin (Sigma-Aldrich, St Louis, MO, USA; Sigma-Aldrich, 2015). A 1 g of meat sample was homogenized with 10 mL of 20mM potassium phosphate buffer at pH 6.5 containing 0.6M sodium chloride at 12800 rpm with an IKA® T18 digital Ultra-Turrax® (IKA-Werke GmgH & Co., Germany) disperser in an ice bath for 15 sec. The homogenate was centrifuged at 4000 × g for 2 min and the supernatant used for analyses. A 100 µL of 2,4-dinitrophenylhydrazine (DNPH) was added to 100 µL of supernatant and incubated at room temperature for 10 min. One hundred microliters of 100% trichloroacetic acid was further added and incubated on ice for 5 min. The DNPH was washed off from the pellet twice with 500 µL of ice-cold acetone followed by centrifugation at 13000 × g for 2 min. The pellet was finally resolubilized in 200 µL of 6M Guanidine solution. The same procedure was conducted using 2M hydrochloric acid per sample and designated as blank. The absorbance of the supernatant was measured at 375 using a micro-plate reader (SPECTROstar Nano, Ortenberg, Germany). The blank was subtracted from their corresponding values. The carbonyl content was calculated as nmol carbonyl/ mg protein calculated using a molar extinction coefficient of 22 mM^{-1}/ cm.

7.4.3 Microbial analyses

Ten grams of meat were placed into aseptic stomacher bags using sterile tweezers. Each sample was homogenized in a 90 ml 0.02% peptone water buffered (w/ v) using a BagMixer® 400

(Interscience International, 78860 St Nom, France) for 2 min. Four-fold serial dilutions of the homogenate were prepared in duplicate. Total viable counts (TVC) were determined on 3M™ Petrifilm™ Aerobic Count Plates (3M, USA) incubated at 35 ± 1 °C for 48 ± 2 h. *Escherichia coli* (*E. coli*) and coliforms were determined on 3M™ Petrifilm™ *E. coli* and Coliform count plates (3M, USA) incubated at 35 ± 1 °C for 24 ± 2 h (AOAC, method; 998.08). *Enterobacteriaceae* counts were determined on 3M™ Petrifilm™ *Enterobacteriaceae* Count Plate (3M, USA) incubated at 37 ± 1 °C for 24 ± 2 h (AOAC, method; 2003.01). All microbial counts were expressed as $\log_{10}$ of colony forming units/ g of fresh meat (log CFU/ g meat).

7.4.4 Sensory quality evaluation

The frozen right LTL samples were thawed overnight at 4 °C. The *longissimus thoracis* was used for the training panel and the LL for the testing phase. Subcutaneous fat were removed from each loin and descriptive sensory analyses performed on both fractions as described by Erasmus et al. (2016). An eleven-member trained panelist was used over eight sessions with three loins per session randomized with respect to presentation order. Panelists were allocated individual tasting booths fitted with computers with the Compusense five® (Compusense, Guelph, Canada) software program. Aroma attributes for meat and subcutaneous fat, and palate and textural attributes were evaluated on the meat (Table 7.2).

7.4.5 Statistical analysis

For microbial counts and sensory scores, the Shapiro-Wilk test was performed using PROC UNIVARIATE (SAS, 2012) to test for normality of the residuals. Data that did not follow a normal distribution was transformed using $\log_{10}$ transformation. Data for shelf stability were subjected to repeated measures' analysis using the GLIMMIX procedures of SAS (2012). Using a first-order autoregressive covariance structure, the model incorporated the fixed effects of diet, day and diet

× day interaction using the following model: $y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \varepsilon_{ijk}$, where y is the observation (i.e., color descriptors, FRAP, lipid and protein oxidation and microbial load) at time k on the j^{th} subject assigned to i^{th} diet; μ is the mean for the i^{th} diet at time k; α_I is the fixed effect of the i^{th} diet; β_j is the fixed effect of the j^{th} day, $(\alpha\beta)_{ij}$ is the diet × day interaction effect and ε_{ijk} is the random error. Day was included in the model as the repeated factor and lamb as the random factor.

Sensory data were analyzed using response surface regression (SAS, 2012) with the following model: $y = \beta_0 + \beta_1 x + \beta_2 x^2 + \varepsilon$, where y is the observation (sensory attribute), β_0, β_1, and β_2 are the regression coefficients and x is the inclusion level of sun-dried GP meal and ε is the random error. Treatment means were generated using least-square means procedure of SAS (2012) and the Tukey's adjustment for multiple comparisons was adopted. Probability values ≤ 0.05 were considered significant and a tendency was reported if $0.05 < P \leq 0.10$.

Table 7.2 Descriptive attributes and reference standards used for the evaluation of the intensity ratings of the sensory attributes for lamb meat and subcutaneous fat

	ATTRIBUTES	Description of attributes	Reference standards
Aroma and palate attributes: Lamb meat and subcutaneous fat	Lamb Fat	Roasted lamb fat aroma and flavor	Roasted lamb fat
	Lamb Meat	Roasted lamb meat aroma and flavor	Roasted lamb meat with fat
	Herbaceous	Aroma and flavor associated with fresh herbs (i.e., rosemary/ thyme/ coriander/ sage)	Combination of herbs: rosemary, thyme, sage, parsley in water
	Savory broth	Aroma and flavor associated with marmite	5ml of marmite dissolved in 250 mL boiling water
	Sweet-associated	Aroma and flavor associated with the browning of a cooked meat surface (Maillard reaction)	Trimmings of roasted lamb meat with fat
	Metallic	Aroma and flavor associated with raw meat or a blood-like taste	1.6 mg ferrous sulfate/ 1000 mL water
	Barnyard/Kraal	Combination of manure, urine, moldy hay, livestock odors	Combination of manure, urine, hay, collected from dairy, horse barn
	Rancid	Aroma or flavor associated with oxidation compounds derived from fat	
	Stale Fat	Aroma associated with fermented or spoiled notes	
Texture attributes: lamb meat	Initial juiciness	Amount of fluid exuded when pressed between thumb, forefinger.0 = Dry, 100 = Juicy	
	Sustained juiciness	Impression formed after first 5 chews using the molar teeth.0 = Dry, 100 = Juicy	
	Tenderness	Impression of tenderness after the first 5 chews using molar teeth.0 = Tough, 100 = Tender	
	Residue	Amount of residue left in mouth after 5 chews using molar teeth.0 = None, 100 = Abundant	

7.5 Results

7.5.1 Chemical composition of experimental diets

All the chemical composition parameters of all diets are shown in Table 7.1. The energy and protein contents were similar across diets. However, ether extract, lignin, non-fiber carbohydrates, total tannins and proanthocyanidins increased with addition of GP to the diet. Neutral detergent fiber declined with increasing levels of GP in the diet.

7.5.2 Color stability of lamb meat supplemented with GP under retail display

Neither diet nor diet × day interaction influenced any of the color descriptors ($P > 0.05$). Day, however, affected lightness (L*); redness (a*) and yellowness (b*) ($P \leq 0.05$; Fig. 7.1). Overall, L* increased over time with d 9 having the highest values ($P \leq 0.05$; Fig. 7.1a). Redness values increased ($P \leq 0.05$; Fig. 7.1b) from d 1 to d 3 and thereafter declined to day 9. The b* values increased ($P \leq 0.05$) from d 1 to d 3 and then plateaued to d 9 (Fig. 7.1c).

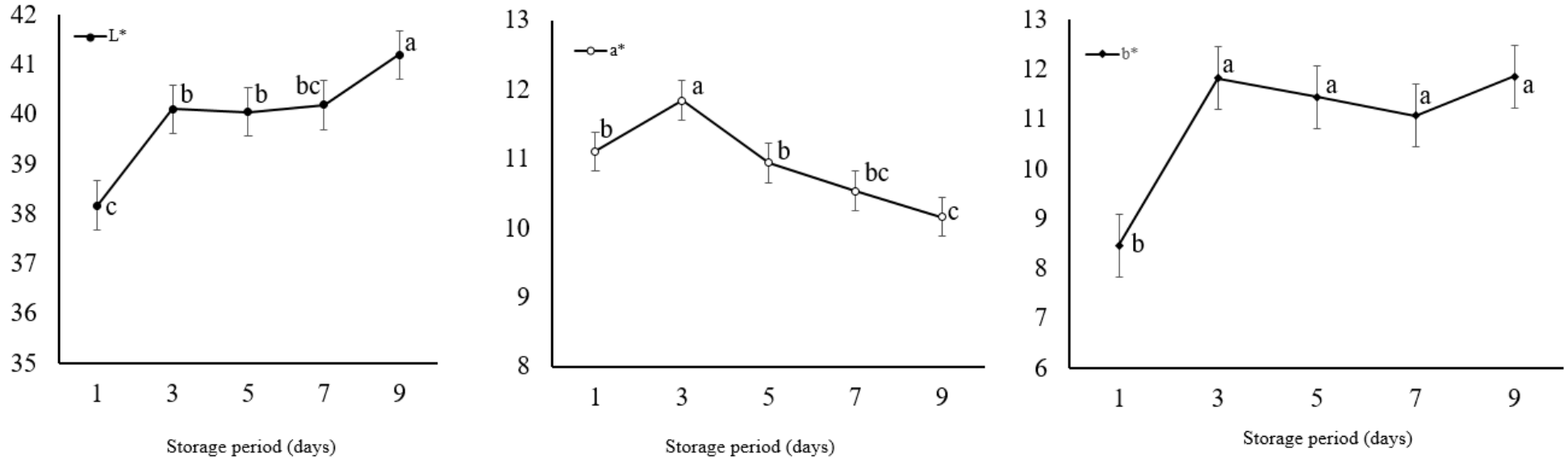


Figure 7.1 Effect of grape pomace on meat color (L*, a* and b*) parameters of retail displayed lamb meat under

7.5.3 Antioxidant activity, lipid and protein oxidation of lamb meat supplemented with GP in retail display

Diet × day interaction had an influence on the antioxidant activity ($P \leq 0.05$). From d 1 to d 7, the 15 and 20% GP diets had higher ($P \leq 0.05$; Fig. 7.2) antioxidant activities than the 0 and 5% GP diets. The 10% GP antioxidant activity was similar ($P > 0.05$) to that of 0 and 5% GP diets from d 1 to 3, and but from d 7 to d 9, it was similar ($P > 0.05$) to the 15 and 20% GP diets.

Interactive effects of diet and day were significant ($P \leq 0.05$). From d 1 to 3, all the diets had no differences in TBARS ($P > 0.05$). A similar trend was observed for 0, 5 and 10% dietary treatments from d 5 to d 9 ($P > 0.05$). The 20% GP-based diet, however, had lower ($P \leq 0.05$; Fig. 7.3) TBARS values between d 5 and 9 relative to the 0 and 5% GP diets.

The results for carbonyl content are shown in Table 7.4. Interactive effects between diet and day were significant ($P \leq 0.05$). Relative to other diet × day interaction, the control diet on d 7 had the highest carbonyl content ($P \leq 0.05$). Overall, the carbonyl content increased ($P \leq 0.05$) from d 1 to d 7 with the 20% GP diet having the lowest values ($P \leq 0.05$).

7.5.4 Meat microbial load

No *E. coli* was detected on any of the meat samples throughout the shelf life study. None of the microbes assayed showed diet × day interaction ($P > 0.05$). Diet had a significant effect for TVC only ($P \leq 0.05$), with the 20% (3.2 ± 0.14 log CFU/ g meat) GP diet having lower counts than the control diet (3.94 ± 0.14 log CFU/ g meat). The 10% GP diet (3.56 ± 0.14 log CFU/ g meat) was not different ($P > 0.05$) from the 0 and 20 % GP diets. Time in retail display influenced TVC,

coliforms and enterobacteria ($P \leq 0.05$), with d 9 having the highest counts followed by d 7 and d 1, respectively (Figure 7.4; $P \leq 0.05$).

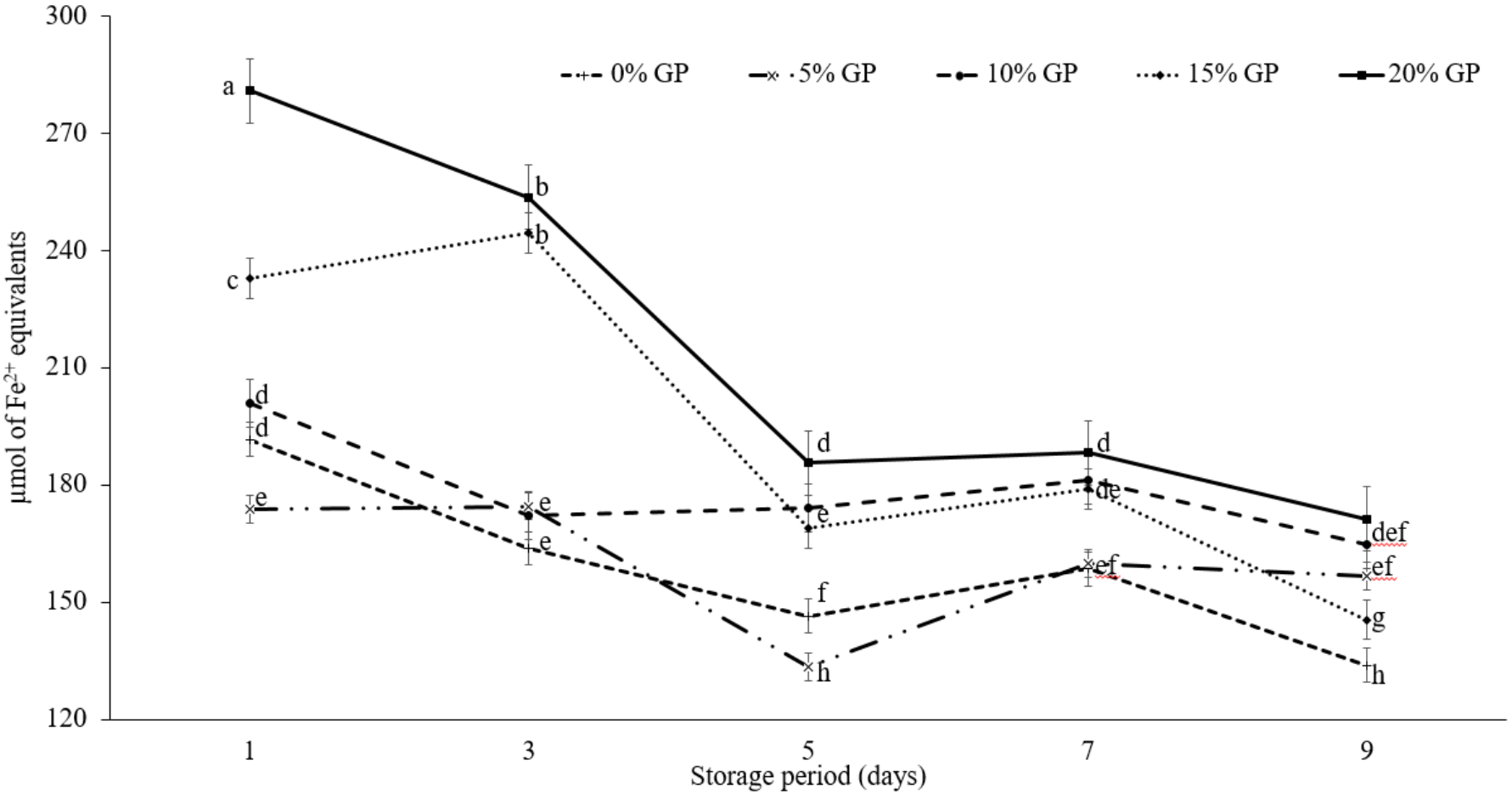


Figure 7.2 Diet × day interactive effects on antioxidative activity of lamb meat under retail display

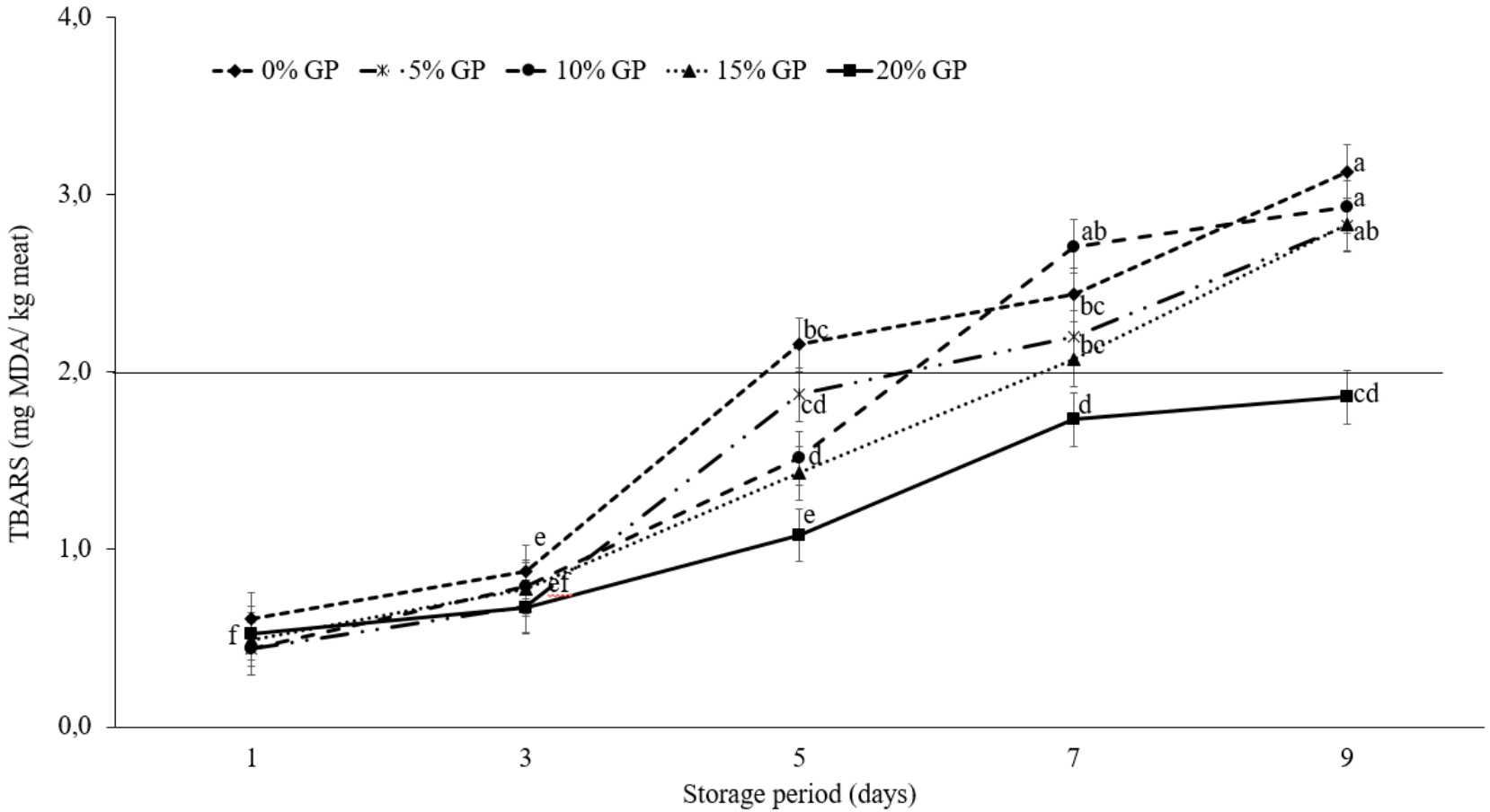


Figure 7.3 Diet × day interactive effects on lipid oxidation of lamb meat under retail display

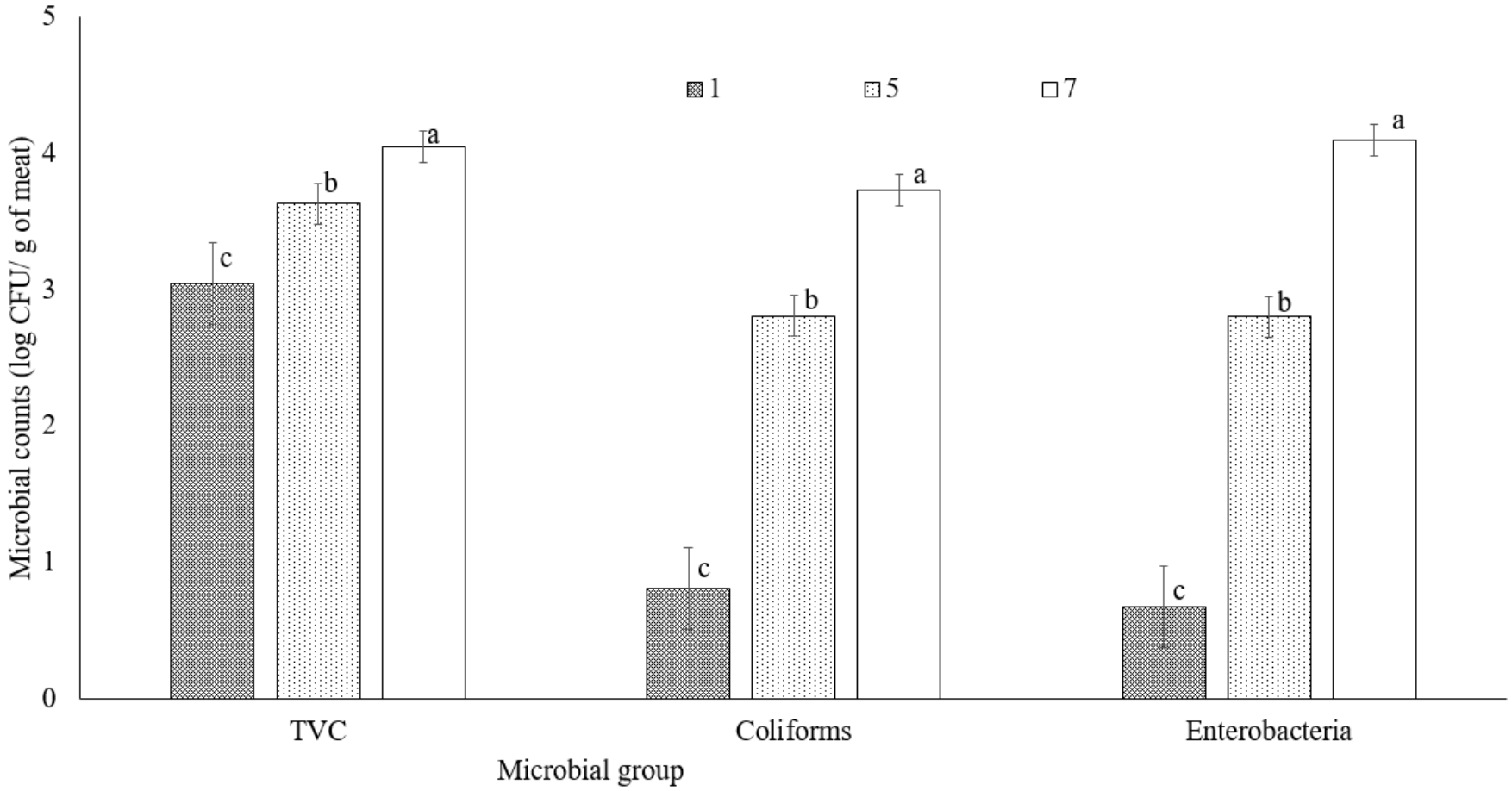


Figure 7.4 Effect of retail display period (day) on microbial counts of grape pomace-enhanced lamb meat

Table 7.3 Effect of dietary red grape pomace on protein oxidation of grape pomace-enhanced lamb meat under retail display

Item	Day	Inclusion of grape pomace (g GP/ kg diet)			SEM[2]	P value		
		0	100	200		Diet	Day	Diet × day
Carbonyl content[1]	1	1.43^{e}	1.06^{e}	1.05^{e}	0.199	<0.0001	<0.0001	0.021
	5	4.00^{c}	3.60^{cd}	3.42^{d}				
	7	6.52^{a}	5.10^{b}	4.95^{b}				

[1] Carbonyl content expressed as nmol carbonyl/ mg protein.
[2] SEM: standard error of the mean.
$^{a-d}$ Least square means for each diet × day interaction with different superscript letters are different ($P \leq 0.05$).

7.5.5 Sensory quality of grape pomace finished lamb meat

The effect of GP inclusion on sensory attributes of lamb meat are presented in Table 7.4. Lamb metallic flavor decreased linearly ($P \leq 0.05$) with addition of GP to the diet. The rest of sensory quality attributes were not affected by the addition of GP ($P > 0.05$).

Table 7.4 Sensory quality of red grape pomace finished lamb meat and subcutaneous fat sensory attributes

Item	Attributes	Inclusion of grape pomace (g/ kg)			SEM[a]	P value		
		0	100	200		Diet	Linear	Quadratic
Subcutaneous fat	Lamb fat aroma	73.8	74.2	72.9	0.84	0.467	0.405	0.365
	Lamb meat aroma	70.1	70.5	70.2	1.04	0.963	0.940	0.793
	Savory broth aroma	16.3	16.5	16.2	0.33	0.694	0.693	0.453
	Sweet-associated aroma	20.8	20.5	20.3	0.52	0.776	0.482	0.977
	Stale aroma	3.54	3.87	4.03	0.71	0.866	0.604	0.912
Meat	Lamb fat aroma	14.9	14.8	14.9	0.33	0.949	0.898	0.768
	Lamb meat aroma	34.8	34.4	34.3	0.91	0.892	0.664	0.851
	Savory broth aroma	18.5	18.5	18.1	0.54	0.845	0.656	0.715
	Sweet-associated aroma	16.8	17.1	16.8	0.39	0.845	0.958	0.569
	Metallic aroma	34.8	34.4	34.3	0.91	0.892	0.664	0.851
	Lamb fat flavor	31.1	29.9	30.7	0.60	0.545	0.757	0.295
	Lamb meat flavor	76.5	76.3	76.2	0.45	0.873	0.615	0.913
	Savory broth flavor	18.5	18.2	18.3	0.23	0.662	0.488	0.563
	Sweet-associated flavor	20.4	20.6	19.9	0.37	0.455	0.421	0.337
	Metallic flavor	12.4	10.9	10.1	0.82	0.095	0.035	0.655
Meat texture	Initial juiciness	68.0	64.0	64.9	1.93	0.350	0.285	0.327
	Sustained juiciness	77.8	74.9	76.9	1.33	0.432	0.693	0.221
	Tenderness	78.6	76.9	77.6	2.00	0.874	0.774	0.671
	Residue	2.63	1.97	1.53	0.67	0.411	0.190	0.884

[a] SEM: standard error of the mean.

7.6 Discussion

The lack of significant effect of diet on the L*, a* and b* parameters was expected. Similar findings have been reported by Jerónimo et al. (2012) and Zhao et al. (2018) upon supplementing grape seed extract and GP in lamb diets, respectively. The increment in L* values over time (days) can be explained by the structural changes of meat, especially protein denaturation, which results in greater dispersion of light and consequently increase L* (Warris, 2010). The L* values were all above 34, a minimum threshold, below which consumers regard lamb meat visually unacceptable (Khliji et al., 2010). Similarly, the a* were within the recommended value of 19 for lamb meat (Khliji et al., 2010). The observed changes in the a* value up to d 3 could be associated with. However, b* is generally not associated with consumer preference of lamb and is therefore unlikely to have an effect on consumer acceptability (Khliji et al., 2010).The enhanced FRAP values for lamb meat observed for the 20% GP inclusion level up to d 9 compared to the control agrees with earlier studies (Luciano et al., 2011; Moñino et al., 2008) when feeding tanniferous-rich diets. These findings could be linked to the phenolic content and their stability over time (García-Lomillo and González-SanJosé, 2017). Shi et al. (2003) noted that the antioxidant power of proanthocyanidins is 20 times greater than vitamin E and 50 times greater than vitamin C. Immediately post slaughter, there is no supply of antioxidants from the diet, and therefore, the maximum antioxidant is expected, with a decline over time. However, as time progresses, the stability of phenolics declines due to oxidation (García-Lomillo and González-SanJosé, 2017; Tseng and Zhao, 2012) results in the reduced antioxidant activity. This affects the different mechanisms through which phenolics exert their antioxidant activity, such as the scavenging for free radicals, removal of reactive oxygen/ nitrogen species, metal chelation, prevention of auto-oxidation enzymes (e.g., lipoxygenase) (Apak et al., 2016; Riazi et al., 2016). This explains the reduced antioxidant activity towards the end of the retail display. Plant phenolics occur together with a blend of other

antioxidants, and usually form synergistic relationships among the various bioactive compounds, hence, the difficulty to isolate the specific contribution of each compound to the overall antioxidant capacity (Lahmar et al., 2018). However, more reliable results could be obtained through the use of an antioxidant composite potency index score, which is a combination of two or more assays (Apak et al., 2016). This allows the contribution of each mechanism of action (i.e., electron or hydrogen atom transfer systems) exhibited by various bioactive compounds to be accounted for, giving a more conclusive overall oxidative status in the meat (Descalzo et al., 2007). This is important given the inconsistencies in antioxidant activity of polyphenols among several animal species (Descalzo et al., 2007; Sun and Holley, 2012).

The diet × day interaction effect observed for both lipid and protein oxidation is associated with the levels and efficiency of phenolic compounds and their stability over time. Antioxidative capacity has a reciprocal effect with the extent of oxidation in meat. This was observed in the current study, at the beginning of shelf life, the antioxidant activity was high, which corresponded to low oxidation across diets. The lack of differences in the oxidation among diets on d 1 compared to those of d 9 could be due to the residual effects of endogenous antioxidant defense systems such as superoxide dismutase, catalase, and glutathione peroxidase enzymes (Apak et al., 2016; Carocho et al., 2018). As the muscle is converted to meat, the exogenous antioxidant defense system still remains more active relative to the enzyme systems (Carocho et al., 2018), hence the low levels of MDA for the 20% GP on d 9. Oxidative processes in meat are favored by post mortem biochemical changes, with myoglobin oxidation leading to meat discoloration (Giuseppe Luciano et al., 2009). Luciano et al. (2011) reported that tanniferous fed lambs had higher phenolic contents and consequently lower MDA values.

The ability of polyphenols to chelate with metal ion, such as iron, could be a possible explanation for the lower MDA content recorded in the 20% GP diet. Iron is recognized as the

most likely catalyst to promote lipid peroxidation during the Fenton reaction (Apak et al., 2016; Cunha et al., 2018). The lack of an observable trend between the 0 and 5% GP diets could be probably due to the breakdown of MDA to tertiary degradation products during the later stage of storage (Soldatou et al., 2009) because of the absence of or the low levels of phenolics in the 0 and 5% diets, respectively. Another plausible explanation for the higher MDA and carbonyl contents on d 7 and 9 could be related to the oxidizing conditions, such as the permeability of oxygen through the polyvinyl chloride overwrap plastic and the longer time the meat was exposed to light (Bañón et al., 2012). Furthermore, it could be due to the loss of homeostatic control postmortem and the action of pro-oxidant factors on UFA (Morrissey et al., 1998) and/ or side chains of the amino acids (Cunha et al., 2018; Lund et al., 2011). Additionally, it could be possibly emanates from the degradation of active phenolics in GP, especially under high humidity conditions more prevalent in fresh retail displayed meat (Brewer, 2011).

Meat from the 20% GP diet was below the 2 mg MDA/kg, a value regarded as the threshold before development of off flavors (Campo et al., 2006). In contrast, Soldatou et al. (2009) reported that a trained sensory panel was only able to detect rancidity of lamb meat when the TBARS were greater than 4.4 mg MDA/kg. This observation therefore warrants a sensory evaluation to be conducted at different time points to get clarity on such contradictions. The level of TBARS in the present study were generally below that reported by Soldatou et al. (2009), especially for the 20% GP diet, implying that inclusion of dietary GP reduces the level of lipid oxidation, thus increases meat shelf life. The contrast in TBARS in the current study compared to those reported by Campo et al. (2006) and Soldatou et al. (2009) could be attributed to differences in packaging conditions, storage length and the level of antioxidant-prooxidant compounds present in meat (Ponnampalam et al., 2016).

In the current study, the values of carbonyl content were higher than those reported by Santé-Lhoutellier et al. (2008). It is expected that over time the rate of oxidation of biomolecules increases. However, the diet effect was also observed as the GP-based diet tended to have lower protein oxidation, except on day 1. These results do further confirm the protective effect of proanthocyanidin-rich diets against oxidation (Luciano et al., 2011). To our knowledge, no threshold values for carbonyl content in meat has been set. This area of study warrants further research because protein oxidation has negative effects on meat quality decreases bioavailability of amino acids, reduces protein solubility due to polymerization, and impairs on protein digestibility (Falowo et al., 2014; Lund et al., 2011).

The overall decline in TVC with increasing GP in lamb diets might be linked to the antimicrobial properties of polyphenols (Apak et al., 2016). Catechins, for example, have been shown to be highly potent inhibitors against DNA gyrase enzyme, (Khan et al., 2018). Other mechanisms exhibited by polyphenols on microbes include the inhibition of biofilms, chelation of metal ions needed for energy production and/ or alteration of the phospholipid bilayer, subsequently increasing the leakage of vital components required for the survival of bacteria (Papuc et al., 2017; Sun and Holley, 2012). It is worth noting that for the 9 days, TVC did not surpass the 7 log CFU/g, a microbial acceptable threshold value beyond which meat is deemed unsafe for human consumption (Soldatou et al., 2009). These values are in accordance to findings by Guerra-Rivas et al. (2016) who supplemented lambs with GP, vitamin E and grape seed extract. The findings that all microbes increase over time is expected as phenolics degrade with time, so do their protective effects (García-Lomillo and González-SanJosé, 2017).

The lack of differences among sensory attributes among the treatments could have been caused by a similar fatty acid profile among diets. Phenolics modulate ruminal fatty acid biohydrogenation (Morales and Ungerfeld, 2015). However, the levels in the diets were probably

not high enough to have a significant effect on rumen microbes responsible for the process. We could not an immediate explanation with regards to the linear decline in the metallic flavor across diets. The observation that GP had minimal effects on meat sensory quality agree with previous studies with other natural antioxidants (Bellés et al., 2017; Chaves et al., 2008; González-Ríos et al., 2016). The inclusion of GP in lamb diets did not result in undesirable effects on the organoleptic properties of meat. This aspect is important because consumers tend not to purchase lamb meat with uncharacteristic aromas and flavors.

7.7 Conclusions

The 20% GP-based diet improved the shelf life of lamb meat through the enhancement of the antioxidant activity and reduction of TVC, lipid and protein oxidation. Overall, the inclusion of GP did not bring about unusual flavors or odors in any of the meat samples. Further studies are warranted to investigate the eating quality up to d 9 since the TBARS were below those recommended for the development of rancidity in lamb. Inclusion of 20% GP in lamb finishing diets as a natural and sustainable source of polyphenols may represent a novel strategy to valorize winery wastes as feed supplements for livestock to meet both meat industry and consumers requests for natural preservatives in meat.

7.8 References

Andrés, A.I., Petrón, M.J., Adámez, J.D., López, M., Timón, M.L., 2017. Food by-products as potential antioxidant and antimicrobial additives in chill stored raw lamb patties. Meat Sci. 129, 62–70.

Apak, R., Özyürek, M., Güçlü, K., Çapanoɨlu, E., 2016. Antioxidant activity/capacity measurement. 1. Classification, physicochemical principles, mechanisms, and electron transfer (ET)-based assays. J. Agric. Food Chem. 64, 997–1027.

Ávila-Gálvez, M.Á., García-Villalba, R., Martínez-Díaz, F., Ocaña-Castillo, B., Monedero-Saiz, T., Torrecillas-Sánchez, A., Abellán, B., González-Sarrías, A., Espín, J.C., 2019. Metabolic profiling of dietary polyphenols and methylxanthines in normal and malignant mammary tissues from breast cancer patients. Mol. Nutr. Food Res. 1801239.

Bañón, S., Méndez, L., Almela, E., 2012. Effects of dietary rosemary extract on lamb spoilage under retail display conditions. Meat Sci. 90, 579–583.

Bedale, W., Sindelar, J.J., Milkowski, A.L., 2016. Dietary nitrate and nitrite: Benefits, risks, and evolving perceptions. Meat Sci. 120, 85–92. https://doi.org/10.1016/j.meatsci.2016.03.009

Bellés, M., Alonso, V., Roncalés, P., Beltrán, J.A., 2017. Effect of borage and green tea aqueous extracts on the quality of lamb leg chops displayed under retail conditions. Meat Sci. 129, 153–160.

Brand, T.S., Van Der Westhuizen, E.J., Van Der Merwe, D.A., Hoffman, L.C., 2017. Effect of days in feedlot on growth performance and carcass characteristics of Merino, South African Mutton Merino and Dorper lambs. S. Afr. J. Anim. Sci. 47, 26–33.

Brewer, M.S., 2011. Natural antioxidants: Sources, compounds, mechanisms of action, and potential applications. Compr. Rev. Food Sci. Food Saf. 10, 221–247.

Campo, M.M., Nute, G.R., Hughes, S.I., Enser, M., Wood, J.D., Richardson, R.I., 2006. Flavour perception of oxidation in beef. Meat Sci. 72, 303–311.

Carocho, M., Morales, P., Ferreira, I.C.F.R., 2018. Antioxidants: Reviewing the chemistry, food applications, legislation and role as preservatives. Trends Food Sci. Technol. 71, 107–120.

Chaves, A. V., Stanford, K., Gibson, L.L., McAllister, T.A., Benchaar, C., 2008. Effects of carvacrol and cinnamaldehyde on intake, rumen fermentation, growth performance, and

carcass characteristics of growing lambs. Anim. Feed Sci. Technol. 145, 396–408.

Chibane, B.L., Degraeve, P., Ferhout, H., Bouajila, J., Oulahal, N., 2019. Plant antimicrobial polyphenols as potential natural food preservatives. J. Sci. Food Agric. 99, 1457–1474.

Chikwanha, O.C., Muchenje, V., Nolte, J.E., Dugan, M.E.R., Mapiye, C., 2019. Grape pomace (Vitis vinifera L. cv. Pinotage) supplementation in lamb diets: Effects on growth performance, carcass and meat quality. Meat Sci. 147, 6–12.

Chikwanha, O.C., Raffrenato, E., Muchenje, V., Musarurwa, H.T., Mapiye, C., 2018. Varietal differences in nutrient, amino acid and mineral composition and in vitro rumen digestibility of grape (Vitis vinifera) pomace from the Cape Winelands vineyards in South Africa and impact of preservation techniques. Ind. Crops Prod. 118, 30–37.

Cunha, L.C.M., Monteiro, M.L.G., Lorenzo, J.M., Munekata, P.E.S., Muchenje, V., de Carvalho, F.A.L., Conte-Junior, C.A., 2018. Natural antioxidants in processing and storage stability of sheep and goat meat products. Food Res. Int. 111, 379–390.

Descalzo, A.M., Insani, E.M., Biolatto, A., Sancho, A.M., García, P.T., Pensel, N.A., Josifovich, J.A., 2005. Influence of pasture or grain-based diets supplemented with vitamin E on antioxidant/oxidative balance of Argentine beef. Meat Sci. 70, 35–44.

Descalzo, A.M., Rossetti, L., Grigioni, G., Irurueta, M., Sancho, A.M., Carrete, J., Pensel, N.A., 2007. Antioxidant status and odour profile in fresh beef from pasture or grain-fed cattle. Meat Sci. 75, 309–317.

Eisler, M.C., Lee, M.R.F., Tarlton, J.F., Martin, G.B., Beddington, J., Dungait, J.A.J., Greathead, H., Liu, J., Miller, H., Misselbrook, T., Murray, P., Vinod, V.K., Van Saun, R., Winter, M., 2014. Agriculture: Steps to sustainbale livestock. Nature 507, 32–34.

Erasmus, S.W., Hoffman, L.C., Muller, M., van der Rijst, M., 2016. Variation in the sensory profile of South African Dorper lamb from extensive grazing systems. Small Rumin. Res. 144, 62–74.

Estévez, M., Luna, C., 2017. Dietary protein oxidation: A silent threat to human health? Crit. Rev. Food Sci. Nutr. 57, 3781–3793.

Falowo, A.B., Fayemi, P.O., Muchenje, V., 2014. Natural antioxidants against lipid-protein oxidative deterioration in meat and meat products: A review. Food Res. Int. 64, 171–181.

FAO, 2019. SAVE FOOD: Global Initiative on Food Loss and Waste Reduction [WWW Document]. URL http://www.fao.org/save-food/resources/keyfindings/infographics/meat/en/ (accessed 2.21.19).

García-Lomillo, J., González-SanJosé, M.L.M.L., 2017. Applications of wine pomace in the food industry: Approaches and functions. Compr. Rev. Food Sci. Food Saf. 16, 3–22.

Gatellier, P., Mercier, Y., Juin, H., Renerre, M., 2005. Effect of finishing mode (pasture- or mixed-diet) on lipid composition, colour stability and lipid oxidation in meat from Charolais cattle. Meat Sci. 69, 175–186.

González-Ríos, H., Dávila-Ramírez, J.L., Peña-Ramos, E.A., Valenzuela-Melendres, M., Zamorano-García, L., Islava-Lagarda, T.Y., Valenzuela-Grijalva, N. V., 2016. Dietary supplementation of ferulic acid to steers under commercial feedlot feeding conditions improves meat quality and shelf life. Anim. Feed Sci. Technol. 222, 111–121.

Gravador, R.S., Luciano, G., Jongberg, S., Bognanno, M., Scerra, M., Andersen, M.L., Lund, M.N., Priolo, A., 2015. Fatty acids and oxidative stability of meat from lambs fed carob-containing diets. Food Chem. 182, 27–34.

Guerra-Rivas, C., Vieira, C., Rubio, B., Martínez, B., Gallardo, B., Mantecón, A.R., Lavín, P., Manso, T., 2016. Effects of grape pomace in growing lamb diets compared with vitamin E and grape seed extract on meat shelf life. Meat Sci. 116, 221–229.

Gustavsson, J., Cederbeg, C., Sonesson, U., van Otterdijk, R., Meybeck, A., 2011. Global food losss and food waste: Extent, causes and prevention, in: International Congress Save Food! Food and Agriculture Organization of the United Nations, Rome, Italy, pp. 1–29.

Jerónimo, E., Alfaia, C.M.M., Alves, S.P., Dentinho, M.T.P., Prates, J.A.M., Vasta, V., Santos-Silva, J., Bessa, R.J.B., 2012. Effect of dietary grape seed extract and Cistus ladanifer L. in combination with vegetable oil supplementation on lamb meat quality. Meat Sci. 92, 841–847.

Kafantaris, I., Kotsampasi, B., Christodoulou, V., Makri, S., Stagos, D., Gerasopoulos, K., Petrotos, K., Goulas, P., Kouretas, D., 2018. Effects of dietary grape pomace supplementation on performance, carcass traits and meat quality of lambs. In Vivo (Brooklyn). 32, 807–812.

Khan, T., Sankhe, K., Suvarna, V., Sherje, A., Patel, K., Dravyakar, B., 2018. DNA gyrase inhibitors: Progress and synthesis of potent compounds as antibacterial agents. Biomed. Pharmacother. https://doi.org/10.1016/j.biopha.2018.04.021

Khliji, S., van de Ven, R., Lamb, T.A., Lanza, M., Hopkins, D.L., 2010. Relationship between consumer ranking of lamb colour and objective measures of colour. Meat Sci. 85, 224–229.

Kumar, Y., Yadav, D.N., Ahmad, T., Narsaiah, K., 2015. Recent trends in the use of natural antioxidants for meat and meat products. Compr. Rev. Food Sci. Food Saf. 14, 796–812.

Lahmar, A., Morcuende, D., Andrade, M.J., Chekir-Ghedira, L., Estévez, M., 2018. Prolonging shelf life of lamb cutlets packed under high-oxygen modified atmosphere by spraying

essential oils from North-African plants. Meat Sci. 139, 56–64.

Loureiro, G., Martel, F., 2019. The effect of dietary polyphenols on intestinal absorption of glucose and fructose: Relation with obesity and Type 2 diabetes. Food Rev. Int. 1–17.

Luciano, G., Monahan, F.J., Vasta, V., Biondi, L., Lanza, M., Bella, M., Pennisi, P., Priolo, A., 2009. Lamb meat colour stability as affected by dietary tannins. Ital. J. Anim. Sci. 8, 507–509.

Luciano, G., Monahan, F.J., Vasta, V., Pennisi, P., Bella, M., Priolo, A., 2009. Lipid and colour stability of meat from lambs fed fresh herbage or concentrate. Meat Sci. 82, 193–199.

Luciano, G., Vasta, V., Monahan, F.J., López-Andrés, P., Biondi, L., Lanza, M., Priolo, A., 2011. Antioxidant status, colour stability and myoglobin resistance to oxidation of longissimus dorsi muscle from lambs fed a tannin-containing diet. Food Chem. 124, 1036–1042.

Lund, M.N., Heinonen, M., Baron, C.P., Estévez, M., 2011. Protein oxidation in muscle foods: A review. Mol. Nutr. Food Res. 55, 83–95.

Manso, T., Gallardo, B., Guerra-Rivas, C., 2016. Modifying milk and meat fat quality through feed changes. Small Rumin. Res. 142, 31–37.

Mattos, G.N., Tonon, R. V., Furtado, A.A.L., Cabral, L.M.C., 2017. Grape by-product extracts against microbial proliferation and lipid oxidation: a review. J. Sci. Food Agric. 97, 1055–1064.

Moñino, I., Martínez, C., Sotomayor, J.A., Lafuente, A., Jordán, M.J., 2008. Polyphenols transmission to Segureño lamb meat from ewes' diet supplemented with the distillate from rosemary (Rosmarinus officinalis) leaves. J. Agric. Food Chem. 56, 3363–3367.

Morales, R., Ungerfeld, E.M., 2015. Use of tannins to improve fatty acids profile of meat and milk

quality in ruminants: A review. Chil. J. Agric. Res. 75, 239–248.

Morrissey, P.A., Sheehy, P.J.A., Galvin, K., Kerry, J.P., Buckley, D.J., 1998. Lipid stability in meat and meat products. Meat Sci. 49, S73–S86.

Muela, E., Alonso, V., Campo, M.M., Sañudo, C., Beltrán, J.A., 2014. Antioxidant diet supplementation and lamb quality throughout preservation time. Meat Sci. 98, 289–295.

NRC, 2007. Nutrient Requirements of Small Ruminants. National Academy Press, Washington DC, USA. https://doi.org/10.17226/11654

Papuc, C., Goran, G. V., Predescu, C.N., Nicorescu, V., Stefan, G., 2017. Plant polyphenols as antioxidant and antibacterial agents for shelf-life extension of meat and meat products: Classification, structures, sources, and action mechanisms. Compr. Rev. Food Sci. Food Saf. 16, 1243–1268.

Ponnampalam, E.N., Burnett, V.F., Norng, S., Hopkins, D.L., Plozza, T., Jacobs, J.L., 2016. Muscle antioxidant (vitamin E) and major fatty acid groups, lipid oxidation and retail colour of meat from lambs fed a roughage based diet with flaxseed or algae. Meat Sci. 111, 154–160.

Praet, N., Mathers, C., Hald, T., Devleesschauwer, B., Kirk, M.D., Torgerson, P.R., Bellinger, D.C., de Silva, N.R., Havelaar, A.H., Speybroeck, N., Cawthorne, A., Angulo, F.J., Gargouri, N., Lake, R.J., Gibb, H.J., Stein, C., 2015. World Health Organization global estimates and regional comparisons of the burden of foodborne disease in 2010. PLOS Med. 12, e1001923. https://doi.org/10.1371/journal.pmed.1001923

Riazi, F., Zeynali, F., Hoseini, E., Behmadi, H., Savadkoohi, S., 2016. Oxidation phenomena and color properties of grape pomace on nitrite-reduced meat emulsion systems. Meat Sci. 121,

350–358.

Ribeiro, J.S., Santos, M.J.M.C., Silva, L.K.R., Pereira, L.C.L., Santos, I.A., da Silva Lannes, S.C., da Silva, M.V., 2019. Natural antioxidants used in meat products: A brief review. Meat Sci. 148, 181–188.

Santé-Lhoutellier, V., Engel, E., Aubry, L., Gatellier, P., 2008. Effect of animal (lamb) diet and meat storage on myofibrillar protein oxidation and in vitro digestibility. Meat Sci. 79, 777–783.

SAS Institute Inc., 2012. SAS/STAT User's Guide. Statistics, Version 9.4, 9.2. ed. SAS Institute, Cary, NC: USA.

Shi, J., Yu, J., Pohorly, J.E., Kakuda, Y., 2003. Polyphenolics in grape seeds- biochemistry and functionality. J. Med. Food 6, 291–299.

Sigma-Aldrich, 2015. Protein Carbonyl Content Assay Kit, colorimetric. Technical Bulletin MAK094.

Soladoye, O.P., Juárez, M.L., Aalhus, J.L., Shand, P., Estévez, M., 2015. Protein oxidation in processed meat: Mechanisms and potential implications on human health. Compr. Rev. Food Sci. Food Saf. 14, 106–122.

Soldatou, N., Nerantzaki, A., Kontominas, M.G., Savvaidis, I.N., 2009. Physicochemical and microbiological changes of "Souvlaki" - A Greek delicacy lamb meat product: Evaluation of shelf-life using microbial, colour and lipid oxidation parameters. Food Chem. 113, 36–42.

Sun, X.D., Holley, R.A., 2012. Antimicrobial and antioxidative strategies to reduce pathogens and extend the shelf life of fresh red meats. Compr. Rev. Food Sci. Food Saf. 11, 340–354.

Tseng, A., Zhao, Y., 2012. Effect of different drying methods and storage time on the retention of

bioactive compounds and antibacterial activity of wine grape pomace (Pinot Noir and Merlot). J. Food Sci. 77, H192–H201.

Warner, R.D., McDonnell, C.K., Bekhit, A.E.D., Claus, J., Vaskoska, R., Sikes, A., Dunshea, F.R., Ha, M., 2017. Systematic review of emerging and innovative technologies for meat tenderisation. Meat Sci. 132, 72–89.

Warris, P.D., 2010. Meat Science: an introductory text, 2nd ed. CABI Publishing, Wallingford, UK.

Zhao, J.X., Li, Q., Zhang, R.X., Liu, W.Z., Ren, Y.S., Zhang, C.X., Zhang, J.X., 2018. Effect of dietary grape pomace on growth performance, meat quality and antioxidant activity in ram lambs. Anim. Feed Sci. Technol. 236, 76–85.

Chapter 8 General discussion, conclusions and recommendations

8.1 General discussion

Small stock contributes only 8 – 10% of animal product income in South Africa (Cloete and Olivier, 2010). Unlike goats, most sheep production is undergoing a significant amount of vertical integration, where most of the large feedlots have their own abattoirs. In feedlots, lambs are commonly finished on high-grain diets (Brand et al., 2017; Nkosi and Meeske, 2010). These diets produce meat with high levels of unsaturated fatty acids which react with a range of oxidizing agents predisposing it to oxidation (Cunha et al., 2018; Falowo et al., 2014; Faustman et al., 2010). Although synthetic preservatives have mainly been used to improve the shelf life, their negative effects on human health has reduced their use in the meat industry (Carocho et al., 2018; Cunha et al., 2018; Nikmaram et al., 2018). In this regard, research is being carried out to find novel and naturally occurring bioactive compounds that can preserve the sensory and microbial quality of meat products.

South Africa has a diversity of fruits including grapes, which produce byproducts that are either underutilized or discarded as wastes. These byproducts are promising novel sources of phytochemicals offering new commercial opportunities to the lamb industry as natural preservative strategies to protect and extend the shelf life of lamb meat (Guerra-Rivas et al., 2016; Zhao et al., 2018). The main objective of the current study was to evaluate the potential of red grape pomace (GP) as a feed supplement and meat preservative in lamb production. The main null hypothesis tested was that supplementation of red GP in feedlot diets has no effect on growth performance, carcass attributes, meat quality, shelf life and sensory quality of lamb meat. The potential use of GP by the South African meat industry is limited by the scarcity of information in terms of the nutritional, phenolic content and antioxidant activity.

In Chapter 3, it was hypothesized that dehydration affects the nutrient composition and *in vitro* ruminal neutral digestibility fiber of GP varieties (i.e., Pinotage, Sauvignon Blanc and Shiraz). Overall, sun- and oven-dried Shiraz had higher dry matter (DM), crude protein (CP), neutral detergent fiber (aNDFom), acid detergent fiber (ADFom) and lignin contents, with freeze- and oven-dried Pinotage exhibiting the best mineral composition across the variety × drying interactions. Additionally, freeze-dried Pinotage showed the best amino acid profile and *in vitro* digestibility of aNDFom at 24 and 48 h. It is known that nutrient composition is related to varietal differences among grapes and variation in the drying techniques (Çoklar and Akbulut, 2017). For example, the higher CP observed for red varieties could be related to the influence of pH on proanthocyanidin-protein complexes which occur during the fermentation of red berries, a similar phenomenon occurring in the rumen (Mueller-Harvey, 2006). On the other hand, the low amino acid for thermally-dried pomaces could be attributed to their sensitivity to hot as opposed to freezing conditions (Ong and Law, 2011; Sablani, 2006).

The high ether extract values observed across red variety × drying interactions could have negative implications on fiber digestion efficiency as values between 50 – 70 g/ kg DM are known to disturb rumen digestion (Vahmani et al., 2017). On the contrary, the high aNDFom content in all GP varieties, regardless of drying method makes it a potential alternative fiber source for ruminants (Baumgärtel et al., 2007; Manso et al., 2016; Zepf and Jin, 2013). However, it is important to note that, high fiber content (NDF; >300 g/ kg DM) (Arelovich et al., 2008; Harper and McNeill, 2015; Smith, 2008) in ruminant diets could also limit intake and digestibility as observed for the sun- and oven-dried Shiraz, which had low *in vitro* digestibility values at 24 and 48 h. Overall, GP had high content of phenolics, especially proanthocyanidins, known to have either beneficial or negative consequences depending on their content in the diet. It was, therefore,

important to quantify the impact of dehydration on the retention of bioactive profile of GP and their subsequent biological activities *in vitro*.

The hypothesis tested in Chapter 4 was that dehydration influences the retention of the bioactive compounds and biological activities of GP. The high contents of oleic acid, linoleic acid, total monounsaturated fatty acid (MUFA), total PUFA and phenolics reported for freeze-dried Shiraz compared to other variety × drying interactions could be related to the varietal differences and the impact of dehydration, especially thermal exposure on these biological active compounds (Çoklar and Akbulut, 2017; Ong and Law, 2011; Sablani, 2006). These results concur with a study by Çoklar and Akbulut (2017) who reported that sun (~7 days) and oven (60 °C; 72 h) drying resulted in high reductions of phenolics relative to freeze drying. Larrauri et al. (1997) only observed significant reductions in phenolic content when grape skins were oven-dried at higher temperature regimes of 100 and 140 °C. The current study indicates that antioxidant activity significantly declined with thermal exposure relative to freeze-drying. Although freeze-dried Sauvignon Blanc had the highest proanthocyanidin content and showed the best antioxidant activity, sun- and oven-drying methods showed similar efficiency in the retention of bioactive profiles and the corresponding biological activities. This is of interest to ruminant nutritionists who usually do not consider GP and other fruit byproducts in feed formulations because of the high levels of proanthocyanidins and variable nutrient composition. Current findings suggest that thermal treatments could be used to reduce their levels of proanthocyanidins to the values (<50 g/ kg DM) without compromising the intake and digestibility of feed in ruminants (Mueller-Harvey, 2006; Waghorn, 2008). This recommended proanthocyanidin level was not surpassed by the addition of GP in an *in vitro* digestibility test, but it was observed that bacterial species abundance was reduced with no significant differences in the diversity, evenness and richness. Based on these results, it was important to conduct animal-based trial for the assessment of GP as an ingredient in

ruminant diets. Based on nutrient composition, *in vitro* ruminal aNDFom, bioactive profiles and their retention, it was suggested to select a red variety, that is, Pinotage. This was based on its low proanthocyanidin content and its uniqueness as a locally bred variety.

Chapter 5 tested whether feeding varying levels (0, 100 and 200 g GP / kg of diet DM) of sun-dried Pinotage GP would improve nutrient digestibility, rumen fermentation, microbial nitrogen (N) supply, N retention and utilization efficiency in lambs. Intake of aNDFom and starch decreased linearly while ether extract intake increased linearly with the addition of GP. The binding effect of proanthocyanidins with nutrients is known to have greater preference to CP than NDF (Le Bourvellec and Renard, 2012). A contrast to this phenomenon was observed in the current study. Similar findings were also observed for apparent digestibility values, where the addition of GP in the diets led to reductions of aNDFom digestibility. This could be associated with the negative effects of phenolic compounds contained in GP (Calderón-Cortés et al., 2018), which may affect its feeding value. Similar reductions were also noted for carbohydrate intake, microbial N supply, total purine derivatives excreted and absorbed, but did not adversely affect N retention and utilization efficiency. Such findings could support the argument that GP can be utilized as an ingredient in finishing lambs and thus reduce the level of inclusion of high cost forages, particularly lucerne hay (Ben Salem and Smith, 2008).

Chapter 6 hypothesized that lambs supplemented with increasing levels of sun-dried Pinotage GP would have improved growth performance, carcass traits and meat quality traits. It was observed that dietary treatment exhibited quadratic responses for ADG, live, hot and cold carcass weights with optimum inclusion levels at 96, 97, 122 and 121 g GP / kg of diet DM, respectively. The quadratic response could be explained by the reduction in DMI beyond the optimum values, partly because of the increasing phenolic and lignin contents (Calderón-Cortés et al., 2018; Moore and Jung, 2001; Waghorn, 2008). Polyphenols negatively reduces the rate of fiber digestion by

binding with feed proteins and/ or microbial enzymes, subsequently reduces DMI (Frutos et al., 2004; Makkar, 2003; Waghorn, 2008). The reduction in DMI as a result of proanthocyanidins can also be linked to their astringent taste (Makkar, 2003). The quadratic responses to GP addition reported for hot and cold carcass weights are consistent with differences observed for DMI, final live weight and ADG. Animals having higher DMI and ADG tend to have greater muscle deposition, and consequently high slaughter weights and heavier carcasses (Mapiye et al., 2009). A similar quadratic trend was observed for gross profit analyses, with the heavier animals fetching a higher margin than the lighter ones. Overall, meat quality traits were not negatively affected by GP inclusion. Utilization of GP as a phenolic-rich antioxidant supplement in lamb diets up to 120 g GP / kg of diet DM did not compromise production and meat quality, however, it is crucial to assess the meat shelf stability and the impact it might have on the sensorial quality.

The hypothesis tested in Chapter 7 was that feeding varying levels of sun-dried Pinotage GP would improve shelf stability and sensory quality of lamb meat. Overall, high GP-containing diets were observed to have higher antioxidative power and lower lipid oxidation status during retail display. This was expected as meat with high levels of phenolics tend to have better protective capacity against free radicals responsible for oxidative processes than meat with lower phenolic content (Guerra-Rivas et al., 2016; Riazi et al., 2016). Besides the direct impact of dietary phenolic antioxidants on free radicals, they augment the already existing endogenous antioxidants (Kafantaris et al., 2017; Li and Liu, 2012). The observed decline in microbial populations with increasing GP in lamb diets could also be related to the high contents of phenolics, which are known to induce antimicrobial effect through their destruction on microbial cell membranes and/ or cell walls (Apak et al., 2016). However, the increased microbial loads over time is not uncommon as the effect of antioxidative phenolics decreases with time so do their protective effects (Garrido and Borges, 2013). The lack of differences observation that GP had minimal

effects on meat sensory quality agree with previous studies with other natural antioxidants (Bellés et al., 2017; González-Ríos et al., 2016). This aspect is important considering that tanniferous natural antioxidants may cause an astringent taste, which may result in consumers shunning meat with uncharacteristic aromas and flavors. Overall, based on the current findings, we reject the main hypothesis and conclude that feeding GP between 100 and 120 g GP / kg of diet DM improves animal growth, carcass attributes and meat shelf life without compromising meat physicochemical and sensory quality.

8.2 Conclusions

Overall, Pinotage- and Shiraz-dried interactions had higher nutrient and phenolic profile, except for starch and proanthocyanidins which were higher for Sauvignon Blanc-dried interactions. The higher proanthocyanidin content for Sauvignon Blanc could have contributed to the observed higher antioxidant activity. Both sun and oven drying methods reduced the phenolic contents, while maintaining comparable biological activities relative to the freeze-dried interactions. Freeze-dried interactions had higher *in vitro* NDF digestibility in the order of Pinotage, Shiraz and Sauvignon Blanc. The inclusion of sun-dried red Pinotage GP in lamb diets reduced carbohydrate intake and decreased CP digestibility but rumen digestion parameters were not adversely affected. Grape pomace inclusion up to 120 g GP / kg of diet DM improved growth performance and carcass performance without compromising the physicochemical meat quality parameters. Grape pomace exhibited antioxidative and antibacterial potential at higher inclusion levels, which was accompanied by low levels of lipid and protein oxidation and rate of microbial growth. Based on the current findings, GP may be adopted as a feed ingredient in lamb diets between 100 and g GP / kg of diet DM as a strategy to decrease feeding costs, improve production and shelf life without compromising meat physicochemical quality and sensory quality. However,

because of the variability of the nutrient and phenolic content, it is advisable to always conduct evaluation of these parameters prior to their inclusion in lamb diets.

8.3 Recommendations and further research

Based on the current findings, sun drying could be used as a strategy on a large scale to reduce the content of phenolics which are usually cited as one of the major limitations for the adoption of this winery byproduct. This drying technique was comparable to freeze drying in terms of efficacy at retention of bioactive profiles and biological activities. Furthermore, its low capital input and practicality as there are no infrastructure development when using this drying method. This is more suitable in the Cape Winelands regions, which experience a Mediterranean climate, that is, hot dry summers. This would also assist wineries with extra income from selling dried pomace to feed manufacturers, which is not bulky and cheaper to transport compared to the wet product.

It is further recommended that the inclusion of grape pomace in lamb feedlot diets should not exceed 120 g GP / kg of total mixed rations. This level was observed to have the best growth performance, carcass traits and gross profit margins with no effect on physicochemical meat quality traits. Although above the 120 g GP / kg of diet DM inclusion level, the antioxidative potential was greatest with lower lipid oxidation, except for protein oxidation, these levels could affect productivity. Closer to the optimum inclusion level, antioxidative capacity and lipid oxidation were moderate enough to have beneficial effects on the shelf life of lamb meat. Overall, the inclusion of grape pomace in lamb diets could be used as an alternative feed ingredient because of its low costs, but with the potential to improve production and shelf life without compromising meat physicochemical and sensory qualities.

Aspects that require further research include the following:

1. A comprehensive fatty acid analyses of meat of lambs supplemented with sun-dried Pinotage with emphasis on health beneficial conjugated linoleic acid and their trans-18:1 derivatives. It is known that proanthocyanidins are responsible for modulation of ruminal fatty acids, which are subsequently absorbed and deposited in muscle tissue. This evaluation was not conducted in the present study owing to lack of resources that are needed for detailed analysis.
2. Quantification of dietary phenolics and/ or their metabolites that are absorbed and eventually absorbed and deposited in the muscle tissue.
3. Investigate the effect of supplementing different grape pomace varieties on lamb production and meat quality. This is important considering observed differences biological profiles between the white and red varieties.
4. Future research should focus on the use of replacing one single ingredient with grape pomace versus the extensive replacement as is currently practiced in commercial feed industry.

8.4 References

Apak, R., Özyürek, M., Güçlü, K., Çapanoɨlu, E., 2016. Antioxidant activity/capacity measurement. 1. Classification, physicochemical principles, mechanisms, and electron transfer (ET)-based assays. J. Agric. Food Chem. 64, 997–1027.

Arelovich, H.M., Abney, C.S., Vizcarra, J.A., Galyean, M.L., 2008. Effects of dietary neutral detergent fiber on intakes of dry matter and net energy by dairy and beef cattle : Analysis of published data. Prof. Anim. Sci. 24, 375–383.

Baumgärtel, T., Kluth, H., Epperlein, K., Rodehutscord, M., 2007. A note on digestibility and energy value for sheep of different grape pomace. Small Rumin. Res. 67, 302–306.

Bellés, M., Alonso, V., Roncalés, P., Beltrán, J.A., 2017. Effect of borage and green tea aqueous

extracts on the quality of lamb leg chops displayed under retail conditions. Meat Sci. 129, 153–160.

Ben Salem, H., Smith, T., 2008. Feeding strategies to increase small ruminant production in dry environments. Small Rumin. Res. 77, 174–194.

Brand, T.S., Van Der Westhuizen, E.J., Van Der Merwe, D.A., Hoffman, L.C., 2017. Effect of days in feedlot on growth performance and carcass characteristics of Merino, South African Mutton Merino and Dorper lambs. S. Afr. J. Anim. Sci. 47, 26–33.

Calderón-Cortés, J.F., González-Vizcarra, V.M., Pétriz-Celaya, Y., Pujol, L.C., Barreras, A., Plascencia, A., 2018. Energy value of unfermented dried grape pomace as substitute of alfalfa hay in diets for growing lambs. Aust. J. Vet. Sci. 63, 59–63.

Carocho, M., Morales, P., Ferreira, I.C.F.R., 2018. Antioxidants: Reviewing the chemistry, food applications, legislation and role as preservatives. Trends Food Sci. Technol. 71, 107–120.

Cloete, S.W., Olivier, J.J., 2010. South African sheep and wool Industry, in: International Sheep and Wool Handbook. pp. 95–112.

Çoklar, H., Akbulut, M., 2017. Effect of sun, oven and freeze-drying on anthocyanins, phenolic compounds and antioxidant activity of Black grape (Ekşikara) (*Vitis vinifera* L.). South African J. Enol. Vitic. 38, 264–272.

Cunha, L.C.M., Monteiro, M.L.G., Lorenzo, J.M., Munekata, P.E.S., Muchenje, V., de Carvalho, F.A.L., Conte-Junior, C.A., 2018. Natural antioxidants in processing and storage stability of sheep and goat meat products. Food Res. Int. 111, 379–390.

Falowo, A.B., Fayemi, P.O., Muchenje, V., 2014. Natural antioxidants against lipid-protein oxidative deterioration in meat and meat products: A review. Food Res. Int. 64, 171–181.

Faustman, C., Sun, Q., Mancini, R., Suman, S.P., 2010. Myoglobin and lipid oxidation interactions: Mechanistic bases and control. Meat Sci. 86, 86–94.

Frutos, P., Hervás, G., Mantecón, A.R., 2004. Tannins and ruminant nutrition. Spanish J. Agric. Res. 2, 191–202.

Garrido, J., Borges, F., 2013. Wine and grape polyphenols - A chemical perspective. Food Res. Int. 54, 1844–1858.

González-Ríos, H., Dávila-Ramírez, J.L., Peña-Ramos, E.A., Valenzuela-Melendres, M., Zamorano-García, L., Islava-Lagarda, T.Y., Valenzuela-Grijalva, N. V., 2016. Dietary supplementation of ferulic acid to steers under commercial feedlot feeding conditions improves meat quality and shelf life. Anim. Feed Sci. Technol. 222, 111–121.

Guerra-Rivas, C., Vieira, C., Rubio, B., Martínez, B., Gallardo, B., Mantecón, A.R., Lavín, P., Manso, T., 2016. Effects of grape pomace in growing lamb diets compared with vitamin E and grape seed extract on meat shelf life. Meat Sci. 116, 221–229.

Harper, K., McNeill, D., 2015. The role iNDF in the regulation of feed intake and the importance of its assessment in subtropical ruminant systems (the role of iNDF in the regulation of forage intake). Agriculture 5, 778–790.

Kafantaris, I., Kotsampasi, B., Christodoulou, V., Kokka, E., Kouka, P., Terzopoulou, Z., Gerasopoulos, K., Stagos, D., Mitsagga, C., Giavasis, I., Makri, S., Petrotos, K., Kouretas, D., 2017. Grape pomace improves antioxidant capacity and faecal microflora of lambs. J. Anim. Physiol. Anim. Nutr. (Berl). 101, e108–e121.

Larrauri, J.A., Rupérez, P., Saura-Calixto, F., 1997. Effect of drying temperature on the stability of polyphenols and antioxidant activity of red grape pomace peels. J. Agric. Food Chem. 45, 1390–1393.

Le Bourvellec, C., Renard, C.M., 2012. Interactions between polyphenols and macromolecules quantification methods and mechanisms. Crit. Rev. Food Sci. Nutr. 52, 213–248.

Li, Y., Liu, S., 2012. Reducing lipid peroxidation for improving colour stability of beef and lamb:

On-farm considerations. J. Sci. Food Agric. 92, 719–726.

Makkar, H.P.S., 2003. Effects and fate of tannins in ruminant animals, adaptation to tannins, and strategies to overcome detrimental effects of feeding tannin-rich feeds. Small Rumin. Res. 49, 241–256.

Manso, T., Gallardo, B., Guerra-Rivas, C., 2016. Modifying milk and meat fat quality through feed changes. Small Rumin. Res. 142, 31–37.

Mapiye, C., Chimonyo, M., Dzama, K., Strydom, P.E., Muchenje, V., Marufu, M.C., 2009. Nutritional status, growth performance and carcass characteristics of Nguni steers supplemented with Acacia karroo leaf-meal. Livest. Sci. 126, 206–214.

Moore, K.J., Jung, H.-J.G., 2001. Lignin and fiber digestion. J. Range Manag. 54, 420–430.

Mueller-Harvey, I., 2006. Unravelling the conundrum of tannins in animal nutrition and health. J. Sci. Food Agric. 86, 2010–2037.

Nikmaram, N., Budaraju, S., Barba, F.J., Lorenzo, J.M., Cox, R.B., Mallikarjunan, K., Roohinejad, S., 2018. Application of plant extracts to improve the shelf-life, nutritional and health-related properties of ready-to-eat meat products. Meat Sci. 145, 245–255.

Nkosi, B.D., Meeske, R., 2010. Effects of ensiling totally mixed potato hash ration with or without a heterofermentative bacterial inoculant on silage fermentation, aerobic stability, growth performance and digestibility in lambs. Anim. Feed Sci. Technol. 161, 38–48.

Ong, S.P., Law, C.L., 2011. Drying kinetics and antioxidant phytochemicals retention of Salak fruit under different drying and pretreatment conditions. Dry. Technol. 29, 429–441.

Riazi, F., Zeynali, F., Hoseini, E., Behmadi, H., Savadkoohi, S., 2016. Oxidation phenomena and color properties of grape pomace on nitrite-reduced meat emulsion systems. Meat Sci. 121, 350–358.

Sablani, S.S., 2006. Drying of fruits and vegetables: Retention of nutritional/functional quality.

Dry. Technol. 24, 123–135.

Smith, P.S., 2008. Dietary fibre requirements of feedlot lambs. University of the Free State.

Vahmani, P., Rolland, D.C., McAllister, T.A., Block, H.C., Proctor, S.D., Guan, L.L., Prieto, N., López-Campos, Aalhus, J.L., Dugan, M.E.R., 2017. Effects of feeding steers extruded flaxseed on its own before hay or mixed with hay on animal performance, carcass quality, and meat and hamburger fatty acid composition. Meat Sci. 131, 9–17.

Waghorn, G., 2008. Beneficial and detrimental effects of dietary condensed tannins for sustainable sheep and goat production-Progress and challenges. Anim. Feed Sci. Technol. 147, 116–139.

Webb, E.C., Casey, N.H., Van Niekerk, W.A., 1994. Fatty acids in the subcutaneous adipose tissue of intensively fed SA Mutton Merino and Dorper wethers. Meat Sci. 38, 123–131.

Zepf, F., Jin, B., 2013. Bioconversion of grape marc into protein rich animal feed by microbial fungi. Chem. Eng. Process Tech. 1, 1011.

Zhao, J.X., Li, Q., Zhang, R.X., Liu, W.Z., Ren, Y.S., Zhang, C.X., Zhang, J.X., 2018. Effect of dietary grape pomace on growth performance, meat quality and antioxidant activity in ram lambs. Anim. Feed Sci. Technol. 236, 76–85.